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NATIONAL CANCER INSTITUTE

Division of Cancer Cause and Prevention

ANNUAL REPORT

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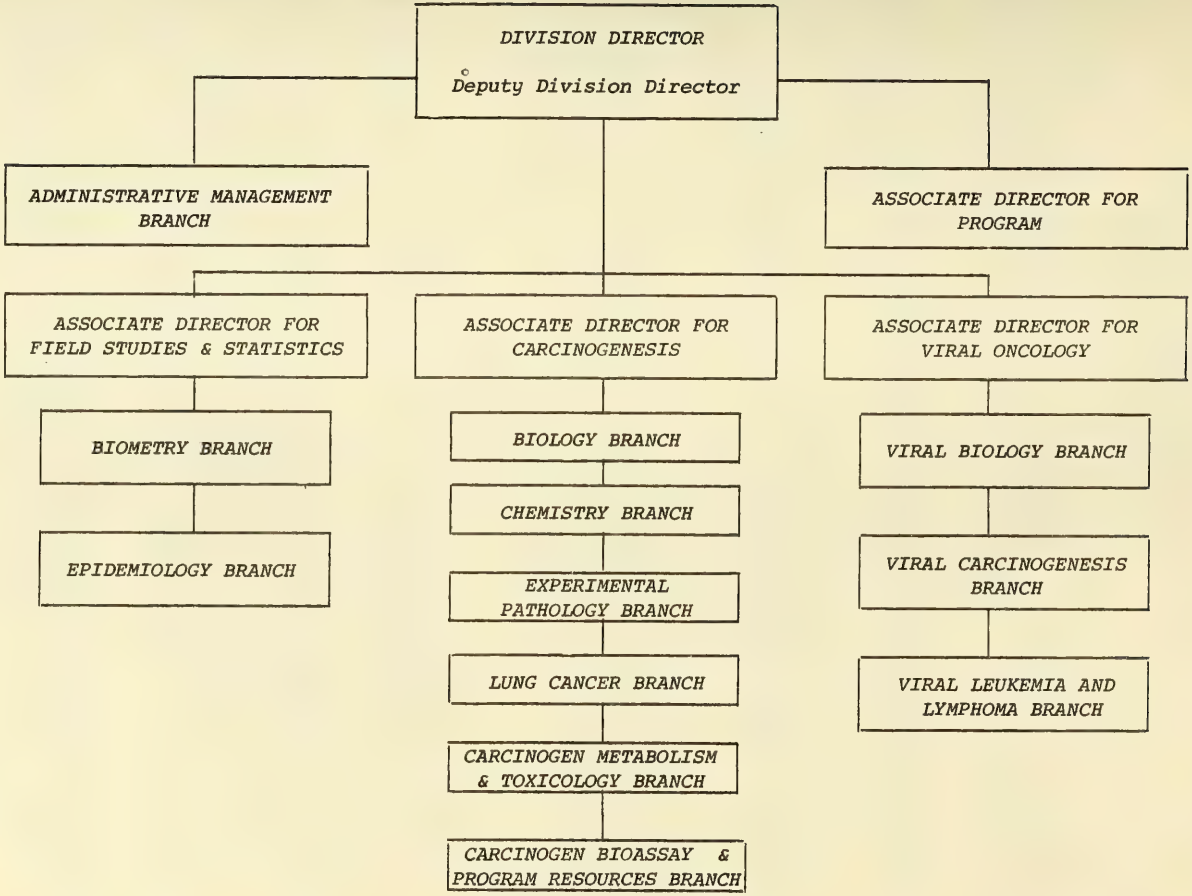
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SUMMARY REPORT
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE
July 1, 1973 - June 30, 1974

The summary report of the Director, Division of Cancer Cause and Prevention, sets forth goals and objectives of the Division, a conceptual base upon which a broad attack on cancer etiology and prevention can be developed, objectives and program approaches to be used for their attainment, and the magnitude of the task and investments required.

A simplified synopsis of major goals, and of pathways to them, is given in Figure 1. More detailed program logic networks for Field Studies and Statistics, Carcinogenesis and Viral Oncology will be found under specific area presentations.

Activities in DCCP are specifically directed towards cancer prevention. Research output is continually utilized in resetting more advanced priorities and in formulating those activities that offer the highest promise of success. The major structures of this scientific management process are indicated in Figure 2.

Newly enacted legislation and funding for the National Cancer Program pose novel challenges and opportunities in research and for cancer control activities in man. This legislation and program recognize the promising advances of cancer research which now make it possible to formulate direct intervention in man's behalf: the definition and engineering of a less hazardous environment and the identification of susceptible individuals.

IMPACT OF CANCER

Cancer occurs in all parts of the world, but variation in the incidence of specific forms of cancer is the rule rather than the exception. A range of ten or twenty-fold is common and for some types of cancer it is far wider.

The mean rate of cancer mortality in the United States occupies an intermediate rank in the worldwide range, but the rank of mortality from specific types of cancer varies markedly. The United States white population has the lowest mortality from cancer of the stomach and close to the highest from cancers of the colon and female breast. As shown in Table 1, the incidence rates of various cancers in the U. S. are expected to increase from a low of 25% each for leukemias and breast cancer to a possible high of 38% for cancer of the pancreas. This table also presents leads towards identification of etiologic factors and towards prevention.

Available evidence suggests that environmental agents and social practices, rather than convential genetic factors, are largely responsible for variation in the occurrence of cancer in different propulations. It should therefore be possible to reduce the occurrence of the major types of cancer in the

United States to the level of the lowest ranking country for that type. Such a reduction would cut mortality from cancer by one-third. This is the minimum goal we should aim for.

More than 52 million Americans now living will eventually have cancer (one in four persons) according to present rates. Cancer will strike over the years in approximately two of three families. In the 1970's in the United States alone, there will be an estimated 3.5 million cancer deaths, 6.5 million new cancer cases, and 10.0 million under medical care for cancer unless the present trends are moderated. Such trends mean that in 1976 there will be 346,000 cancer deaths and 747,000 new cases and in 2000, 471,000 cancer deaths and 1,085,000 new cases.

In the current year there will be near 350,000 cancer deaths in the U. S. or one cancer death nearly every one and one-half minutes. There will be 680,000 new cancer cases and 1,000,000 Americans will be under care for Cancer. The Third National Cancer Survey estimates that the earning income loss due to cancer deaths alone currently deprives our economy of $18 billion annually. This does not include the costs for diagnosis and treatment of the disease. About one-half the cancer cases are below age 65; life expectancy and economic earning power are reduced over a considerable portion of the economically productive ages.

Although cure rates for cancer patients have steadily improved over the past several years, the mortality (both in actual numbers and in rates corrected for population growth and other changes)continues to rise. This is because new cancer cases are appearing at an even greater rate than the improved rate of cures.

PROGRESS, PROBLEMS AND OPPORTUNITIES

A. FIELD STUDIES AND STATISTICS. Cancer can be prevented by identifying its causes and removing them, and/or by increasing the body's defenses against them. Thus, it is important to know not only what causes cancer, but also who is unduly susceptible or resistant. Susceptibility may be immense. For example, if an identical twin develops leukemia during the first year of life, his co-twin is virtually certain to develop leukemia within weeks or months. This increased risk fades quickly with age, and entirely disappears by 6 years of age.

Recently there has been a substantial increase in our list of characteristics of persons who are unusually susceptible to leukemia, lymphoma and other cancers. Once such a list has been created, it can be examined for a common denominator. Many items can be explained by a known or possible genetic defect, sometimes extensive enough to cause various abnormalities in the appearance of chromosomes. It should be noted that the only environmental agent known to be leukemogenic in man, ionizing radiation, and the strongest suspect among the chemicals, benzene, produce long-lasting chromosomal abnormalities in somatic cells.

By contrast, persons at high risk of lymphoma (solid tumors of lymph tissue) have inborn immunological deficiencies (ataxia-telangiectasia, Wiskott-Aldrich

syndrome, or congenital x-linked agammaglobulinemia). Consistent with these observations is the increase reported in the occurrence of lymphomas among persons given immuno-suppressive drugs following renal transplantation. A sudden upturn in one form of lymphoma, Hodgkin's disease, at about 8 years of age, also suggests a relationship to immunological development. Normally, at this age, there is a marked spontaneous involution of lymphoid tissues throughout the child's body. Lymphoid tissues are centers of immunologic activity. It is tempting to think that some failure in this normal regression accounts for the upturn in the occurrence of the neoplasm.

There are many other inborn defects that carry high risk of cancer of various types. In the aggregate, they do not account for a large proportion of cancer but they probably do constitute a large percentage of cancers among young people. In this group, prevention or early detection and treatment can provide decades of healthy life. Peaks soon after birth in the frequency of diagnosis of leukemia, neuroblastoma, Wilms' tumor, and certain forms of brain cancer indicate that many of these neoplasms are likely to have been initiated during intra-uterine life.

Knowledge of these relationships permits physicians to examine children with certain defects more closely than usual, since early detection and prompt removal of these tumors can be life-saving. Scientists, on the other hand, can explore the origins of the tumors in the light of what is known of the associated birth defects. Thus, instead of studying a tumor in terms only of itself, new avenues of approach have been opened. What is learned through these rare mistakes of nature may well apply to prevention of large categories of cancer. In particular, the epidemiologic identification of individuals and populations at increased risk of different cancer patterns aids the investigator in virology and chemical carcinogenesis in searching for specific etiological factors.

International Activities

A contract with NAS-NRC supports and extends cancer research at the Atomic Bomb Casualty Commission in Japan. This has paid off in a modified Japanese attitude toward ABBC, so that there is now substantial involvement of several Japanese universities, including the development of a tissue registry at Hiroshima. These resources provide on-the-ground data resources for the development of epidemiologists. The Japanese Migrant Studies in Hawaii (in cooperation with the Heart and Lung Institute) have provided a very strong suggestion that meat - in particular, beef - is associated with the great increase (4-fold) in cancer of the large bowel in Hawaiian Japanese. This work is being followed up by work in the Carcinogenesis Area in the search for bacterial flora possibly capable of producing carcinogens in the lower bowel.

The field studies with the International Agency for Research on Cancer (IARC) in Iran on cancer of the esophagus have progressed very well. Preliminary results are consistent with a multi-factorial hypothesis consisting of:

(a) the presence of a carcinogen in the food (quite possibly nitrosamines), combined with

(b) possible dietary deficiency, most likely Vitamins A, B_2 and C.

Plans have been developed for appropriate next steps to take to confirm (or deny) this hypothesis. Many other factors have been eliminated, including the use of drugs, hot tea drinking, and dyes used in rug making.

Under the aegis of the UICC, the Epidemiology Branch is collecting data for an international comparison of pediatric cancer around the world. A monthly newsletter is used to communicate important observations on the origins of childhood cancer to pediatric oncologists throughout the world.

The international registry of epidemiology and epidemiologists (as a part of the International Cancer Data Bank) is about to begin operation as a cooperative activity of UICC and NCI.

Work has continued on exploiting special populations in India (the Bombay cancer registry) and in Cali, Colombia (problems of cervical cancer, Hodgkin's disease). Migrant studies of Norwegian and Polish migrants to the United States are being continued - although at a relatively low financial level. Recent work has supported an association between adenomatous polyps and the incidence of colon cancer.

The first tentative steps toward cooperative work with Soviet Russia in cancer epidemiology were taken at a conference in Erevan, Armenian SSR, in January 1974. U. S. data on incidence, mortality and survival, plus some special data on skin cancer have been sent to Soviet Russia.

Domestic Activities

Two major thrusts define domestic studies: data gathering and analysis, and the testing of etiologic hypotheses. With respect to the former, the report of the Third National Cancer Survey (TNCS) was published, for all three years of the survey, and by survey area. Also cancer mortality data, by county, by site, by sex and race for the 20-year period 1950-1969 were published, giving total numbers of cases, and rates. This will be followed by maps, permitting visual identification of clusters. Hospital cost data have been gathered by the TNCS. (The median hospital payments made within two years of diagnosis are between $2000 to $3000). Information on treatments and survival has been reported in the 4th End Results Report. The Third National Cancer Survey and the End Results Program have given way to the SEER (Surveillance, Epidemiology and End Results) Program. Through this program, we hope to develop early data on incidence, mortality and survival, on a grass-roots level, hopefully to provide contemporary data to guide the search for etiologic factors and other control activities.

A special interest in familial and genetic cancers and in childhood cancers continues. Familial cancers (e.g. medullary carcinoma of the thyroid and pheochromocytoma) give an opportunity for effective secondary prevention through frequent examination of family members at high risk. Childhood cancers are being pursued through a national registry of death certificates, a followup of children born in military hospitals where data exist on their intra-uterine exposure to x-irradiation, and in cooperation with the Third National Cancer Survey, will permit study of incidence as well as mortality.

The National Wilm's Tumor Survey has confirmed the high rates of aniridia, and hemihypertrophy in Wilm's tumor children. These are in addition to previously observed high frequency of genito-urinary defects and familial incidence.

Other major areas of work involve studies of prognostic factors, particularly in breast cancer, and Hodgkin's disease. An emphasis on the characteristics of patients who may or may not benefit from certain forms of therapies was developed out of the cooperative studies of cancer of the prostate, undertaken with the Veterans Administration. These studies helped tailor treatments to prostate cancer patients. The concept of the "Epidemiology of Treatment" arose out of these studies and it should develop into a fruitful new field of study.

B. CARCINOGENESIS. The observed relation between exposure to certain chemicals and the development of cancer in man has been historically the first step towards knowledge of the etiology of cancer. A listing of agents presently known to produce cancers in man includes some 10 specific chemicals, 12 mixtures and crude products and, in addition, radioactive materials and radiation (including x-rays and ultraviolet light). The number of items is relatively small because studies capable of identifying an agent as directly causative of cancer in man require large-scale and time consuming epidemiologic studies and have been conducted for a very small proportion of the potential exposures of man. In fact, essentially all materials that have been demonstrated to be carcinogenic for man have also been found to be carcinogenic in animals.

Most chemical carcinogens known from animal studies appear active in a variety of animal species. It is estimated that more than 1000 chemicals have so far been found carcinogenic in animal bioassays out of about 6000 that have been tested.

The major types of cancer in man that can be directly related to known carcinogenic exposures are as follows: (a) cancer of the lung related to cigarette smoke and certain other inhalation hazards (particularly asbestos, chromates, and radioactive materials); (b) cancer of the skin related to exposure to a variety of crude tar products and combustion products as well as to ultraviolet light and other forms of radiation; (c) cancer of the bladder related to exposure to aromatic amines and their derivatives; (d) leukemias related to exposure to radioactive materials; and (e) mesotheliomas related to asbestos exposure. A number of other human cancers have been considered to be related to exposure to environmental carcinogens but the evidence is less clear.

Because of the widespread use of some of these materials, the proportion of the total population exposed and affected is extremely difficult to estimate, with the exception of such materials as cyclamates, DDT and tobacco smoke. It has been estimated that the majority of the present cases of lung cancer in the United States are attributable to cigarette smoking and that factor alone appears to be responsible for a death toll in the neighborhood of 60,000 deaths a year. Current projects to develop less hazardous cigarettes have been expanded by the organization of a special Smoking and Health Program in the Office of the Director, DCCP. Considerable research and newer leads in this area promise to result in decreased risk for those who continue to smoke against all warnings.

The development of our knowledge on the causative factors of cancer in man can be projected along the following lines: (a) epidemiologic studies to correlate specific exposures to certain types of cancer in man with particular emphasis on occupational exposures; (b) additional bioassays in animal models which will identify chemicals as carcinogenic in animals and therefore potentially carcinogenic in man; (c) studies of animal human correlations by comparisons of bioassay results with the results of epidemiologic studies or by bioassays on isolated human tissues by in vitro techniques; and (d) by studies of pharmacologic and metabolic correlations among species.

The major areas of opportunity and need for progress in cancer prevention through studies in Carcinogenesis are: (a) develop better means for the removal of hazardous chemicals from the environment; (b) develop means to enhance the ability of the host to detoxify environmental agents; (c) determine the extent to which chemicals act in synergistic concert with viruses or with physical carcinogens such as irradiation; and (d) develop more rapid and more sensitive means to detect and bioassay the cancer-inducing effects of chemicals for man. The latter area of activity is particularly important. For example, it is estimated that approximately 200,000 new chemicals enter our environment each year. With present assays it takes approximately 2 years and 200 mice at approximately $200 per animal to test 1 chemical. It is hoped that newer studies in animal and human tissue cultures may diminish the 2 years and nearly $40,000 now required to test a single compound. However, until this is achieved the bioassay resources are limited to a few hundred compounds annually and it is necessary to select compounds to be tested on the basis of their distribution in the environment, intake in man and similarity to known carcinogens.

Progress Highlights

1) Bioassay. A contract at the Stanford Research Institute, directed at establishing a ranking of suspicion of carcinogenic hazard for compounds present in man's environment, provides information for the best utilization of current limited bioassay resources and may give early guidelines to the chemical industry, as to the hazards that may be expected by the introduction of a new compound.

The significance of current bioassays for carcinogenicity derives mainly from two considerations, namely, that a) humans in general react like other animals to a variety of insults, the differences being usually quantitative rather than qualitative and b) current bioassay methods, even when performed with adequate biometrical and qualitative controls, can be expected to detect only those compounds that are strongly carcinogenic.

These two reasons prescribe that the greatest attention be given to positive results in current carcinogenicity tests.

Testing and retesting of environmental chemical pollutants at NCI continue to include agricultural chemicals such as pesticides, artificial sweeteners such as cyclamates and saccharin, hormonal compounds generally used as oral contraceptives, and natural products of various molds and fungi. Food preservatives are also under constant scrutiny.

The establishment of standarized, detailed protocols for all the major bioassay procedures and for data acquisition and analyses will enable the Program to make an important transition. Until now, the level of activity was mostly aimed at the screening of environmental chemicals to identify suspected carcinogens for further study. The Program is now preparing to enter into a phase where more emphasis will given to generating detailed bioassay data at the level of sophistication and control necessary to meet regulatory requirements. Emphasis has been placed on the need for documentation of all data entering into and resulting from a carcinogenesis bioassay.

Carcinogenic activity has been identified for several pesticides and pharmaceuticals. Several substituted hydrazines in widespread environmental use have been found to be markedly carinogenic for a variety of organs.

2) Smoking and Health Program. A major effort is under way, under the advice of the Tobacco Working Group, to reduce the risk of the smoker. Current research includes: a) Skin painting and ciliotoxic and cytotoxic bioassays of less hazardous cigarette models. These include cigarettes with various additives, papers of different porosity, reconstituted tobacco sheets of different densities and nature, reconstituted cellulose artificial tobaccos, high and low nicotine tobaccos, tobaccos grown under different fertilization conditions, "puffed" tobaccos, freeze-dried tobaccos, exane extracted tobaccos, etc; b) Chemical characterization and ciliotoxicity bioassays of several experimental cigarette filters; c) Development of novel bioassay methods such as the wax/tar pellet implantation technique in rodent lungs; d)development of inhalation methods in small rodents; e) Determination of nicotine effects by chronic inhalation in dogs; f) Development of experimental methods to measure smoke dose intake in man; g) Pharmacologic and behavioral approaches to demonstrate the feasibility of helping the high-risk smoker through drugs and smoke cessation clinics; h) Epidemiological studies on the health consequences of smoking associated factors, such as demographic and social variations, occupational and environmental characteristics, smoking habits, inhalation characteristics and smoking product used.

Preliminary and tentative results suggest that some cigarette paper types, reconstituted sheets and artificial tobaccos may be desirable for the manufacture of less hazardous cigarettes; however, considerably more research is necessary to duplicate and confirm the initial results, and to establish that improvements related to the carcinogenicity of the condensates do not result in worsening of other characteristics affecting the cardiovascular and respiratory systems. In team with the National Heart and Lung Institute, studies have been developed to determine the cardiovascular and respiratory effects of cigarette smoke in man and experimental animals, for the evaluation of less hazardous smoking products; in team with the United States Department of Agriculture, agronomic tobacco varieties have been tested and may result in less hazardous smoking products. Drugs will be tested that may help the high-risk smoker in counteracting some of the harmful effects of the smoke or in discontinuing the smoking habit. In this area, nicotine antibodies have been successfully produced by scientists under contract with the NCI and may become a significant tool in the treatment of the high risk patient.

3) Development of Bioassay Models. Major programs have been established for the development of biological models for tumor pathogenesis closely correlated to the main types of cancers in man. Lung Cancer and Colon Cancer Programs are well established; Pancreas Cancer and Prostate Cancer Programs, initiated in FY 1972, are now developing programs on Endocrine Cancers and childhood cancers. Their emphasis is on the use of animal models and human material to identify pathogenetic steps susceptible of inhibition or prevention.

In vitro models for neoplastic transformation of cells in culture by chemicals have been developed using cells from hamsters, mice, and guinea pigs. A major advance was the development of a host mediated transformation assay, responsive to carcinogens that require metabolic activation by the host. These new methods are being used to establish their value both as screening techniques and as tools for identifying key steps in carcinogen activation and interaction, susceptible to inhibition. The applicability of these techniques to transformation tests of human cells is currently being investigated. Genetic factors and growth conditioning factors required for neoplastic transformation by chemicals have been identified. Enhancement of chemical cell transformation by radiation and of viral transformation by chemicals have been demonstrated. Transformation of epithelial cells in vitro by chemicals has also been obtained in preliminary experiments. A major advance has been made with the transformation of human cells in vitro by chemical carcinogens, conditioned by corticosteroids.

4) Studies of Carcinogenesis Processes. New emphasis has been given to the study of carcinogen metabolism and toxicology, and a large collaborative effort is underway in the documentation of the conditions of formation and activation of nitrosamines. Pharmacologic inhibition of the formation of carcinogens has been obtained by blocking nitrosation of amines. Other classes of carcinogens are also under study (e.g. hydrazines, metals).

Major metabolic steps in the enzymatic activation of several classes of carcinogens have been identified with special emphasis on polynuclear hydrocarbons. Their reproducibility in human tissues has already been demonstrated in some cases. Micromethods, applicable to determination in small samples of human cells, have been developed for measuring carcinogen activation levels. Inhibitors of carcinogen activation have been identified. The development and use of high-pressure, liquid chromatography has been a milestone in the study of metabolism of polynuclear hydrocarbon carcinogens. This technique can separate as many as ten metabolites of benzo[α]pyrene in a short period of time and can therefore correlate carcinogen susceptibility with unique metabolic profiles.

5) Immunological Studies in Carcinogenesis. Radioimmunoassays have been developed for several carcinogens and related compounds. Progress was made in the study of the components of BCG responsible for tumor cell killing and in the study of the mechanism of action of cell-mediated immunity.

Refined methods have been developed for measurement of serum of carcinoembryonic antigen and rat $alpha_1$-fetoprotein.

C. VIRAL ONCOLOGY. A considerable number of viruses are known which either directly or indirectly cause tumors in different vertebrate species. Some of these replicate in and cause transformation of cultured human cells. The RNA viruses are responsible for many naturally occurring tumors in animals, and this group is most likely to be the cause of some human neoplasms. Certain DNA viruses may also be involved in oncogenesis either as causative agents or as necessary co-factors in cell transformation. Thus far, the only DNA viruses associated with natural cancers of animals and man are herpesviruses. Although candidate viruses have been isolated more frequently in recent years, none as yet has met the rigorous criteria to be considered a human tumor virus. Nevertheless, there is cause to be optimistic, for scientists have acquired the knowledge, techniques, experimental systems, and insight to study this problem. Even if virus particles may not be the direct cause of human cancer, virologists are pursuing the possibility that cellular genes - genes containing information similar to that of RNA or DNA tumor viruses - are expressed in human cancer.

Current knowledge suggests several methods for analysis of human tumors for virus-specific genetic information: (1) Molecular hybridization between cancer cell RNA and natural and synthetic DNA prepared by viral RNA-DNA polymerase; (2) Search for RNA-DNA polymerase activity in human cancers; (3) Search for human virus-specific antigens related to known tumor virus antigens in human cancers; (4) Application of new techniques (e.g. chemical treatment) for the induction of virus or virus genetic information in human tumor cells.

1) RNA Viruses:

Characteristics. RNA tumor viruses possess a number of similar biological and biochemical characteristics, are widely distributed in vertebrate species, and are transmitted vertically under natural circumstances. Infections are often covert, resulting in only partial expression of the viral genome. Each type C virus, regardless of origin, possesses a major internal antigen which permits species delineation (gs-1), and shares a determinant which is interspecies specific (gs-3). There is now good evidence that internal antigens can be detected in embryonic, adult, and tumor tissues of various animal species. This finding is highly significant because the antigen can be found in situations where no virus can be demonstrated.

Of greater importance in terms of understanding the determinants of cancer is the reported linkage between RNA tumor virus and early viral gs antigen expression with the development of cancer late in life.

All RNA tumor viruses contain 60-70S RNA and an enzyme known as RNA-dependent DNA polymerase (RDDP) which permits transcription of viral RNA into DNA. As an antigen, RDDP, like the gs antigen, has both species and interspecies characteristics.

To provide a basis for further understanding information was sought to elucidate the mechanisms involved in the integration of RNA tumor virus information into host cells, and the relationship of this reaction to cell transformation. Recent results indicate the initial product of the RDDP is covalently linked RNA-DNA that is primed for DNA synthesis by a 4S RNA associated with 60-70S RNA. The yield of DNA is also increased by a stimulatory viral protein.

The protein products of virus gene expression in relation to cell transformation are also being studied.

Within the past year progress has been made toward the understanding of the biochemical (molecular) events which occur in the process of cell transformation. These studies are important because they can be applied to human normal and tumor cells to identify virus-specific sequences that have characteristics similar to those of animal RNA tumor viruses. Such studies also help to provide information on mechanisms of virus expression and regulation which could lead to new methods for controlling human cancer at the cellular level.

Analysis of human cancers for evidence of RNA tumor viruses. The powerful tools of molecular hybridization have permitted the analysis of human tumors for viral genetic information. Evidence from animal model systems indicate thus far that every cell line infected with or transformed by RNA tumor viruses possesses virus-specific RNA sequences readily detectable by molecular hybridization. Corresponding neoplasias of murine and human origin were found to exhibit remarkable similarities. For example, human breast carcinomas contained RNA possessing sequence homology to that of murine mammary tumor virus (MMTV).

Following reports of RDDP in the virions of RNA tumor viruses, it was important to determine whether the enzyme is restricted to tumor viruses and whether it is restricted to tumor cells. Attempts are now being made to determine whether the polymerase is present in human cells. RDDP obtained in the particulate fraction (1.16 g/m) of human leukemic cells was purified and characterized. The enzyme could be distinguished from major DNA polymerases of normal cells.

Considerable effort is being directed to search for RNA viruses which might be oncogenic for man. For the past several years sufficient numbers of observations reporting the finding of particles similar to Type C and Type B viruses in human malignancies have been made to conclude that viruses of these types may also infect man.

Following published reports that endogenous animal RNA tumor viruses could be activated by treatment of cultured cells with halogenated uridine, the technique was successfully applied to a human tumor cell culture, and the agent, released in small amounts, is presently undergoing preliminary characterization, both intramurally and within the research contract program.

Attempts to confirm earlier findings showing a correlation between the presence of virus-like particulates in human milk, the RDDP activity associated with fractions separated from the milk, and the breast cancer history of the milk donor have not been successful. A specific inhibitor in milk which interferes with the assay, a ribonuclease, may be responsible for the inconsistent results.

2) DNA Viruses

Characteristics. As recently as 1964, herpesviruses were generally considered to lack oncogenic activity. Prior to that time, the only evidence linking these viruses to animal neoplasias was the finding of herpes-like particles in cells of renal adenocarcinomas of the frog. The discovery of the Epstein-Barr virus (EBV) in cultured lymphoblastoid cells from patients with Burkitt's lymphoma in 1964 stimulated extensive studies to examine herpesviruses as possible etiological agents of cancer in several animal species, including man.

Herpesviruses interact with their host cells in a productive or non-productive manner. During the productive growth cycle, the synthesis of infectious progeny is invariably accompanied by destruction of specific target cells. The non-productive cycle is very similar to that observed with other oncogenic DNA viruses. Stimulation of cellular DNA synthesis, acquisition of virus-induced antigens, incorporation of viral nucleic acid and transformation of normal cells into established lines capable of indefinite proliferation have all been described. Activation of virus synthesis in non-productively infected cells by exposure to mutagens (BUDR, IUDR) or irradiation is usually paralleled by cell death. Herpesviruses, like the RNA tumor viruses, also establish persistent covert infections. Whether latent herpes infections are the result of low-level productive or non-productive interactions has not been determined.

The mechanisms underlying herpes-induced oncogenesis remain obscure. The close association of these viruses with several animal malignancies suggests, but does not prove, their involvement as etiological agents.

Relationship to human cancer. The most outstanding evidence for the existence of human oncogenic herpesviruses is derived from seroepidemiological, biochemical, and biological studies, suggesting an association between various herpesviruses and specific malignant diseases of man. The Epstein-Barr virus is suspect of playing some role in the genesis of Burkitt's lymphoma, nasopharyngeal carcinoma and, to a lesser extent, the sarcomatous form of Hodgkin's disease and chronic lymphocytic leukemia.

The Epstein-Barr virus is closely associated with Burkitt's lymphoma (BL) but the absence of an experimental animal susceptible to infection with this agent has made it difficult to pinpoint its role in the induction of this disease. Therefore, although the evidence which suggests that EBV is the etiological agent of Burkitt's lymphoma is circumstantial at present, it is so strong that a causal rather than a casual relationship is suggested. The tumor is most prevalent in regions of Africa where conditions are extremely favorable for insect-vectored disease. Cases in certain areas have been shown to cluster in time and space.

The establishment of continuous lymphoblastoid cell lines following exposure of lymphocytes from normal donors to EBV again suggests an oncogenic potential for this virus.

A serological association has also been described between EBV and nasopharyngeal carcinoma (NPC) that is as pronounced and consistent as that observed in Burkitt's lymphoma. Virtually all NPC patients have detectable antibody to EBV VCA and MA in high titer. The serologic picture and the demonstration of EBV-DNA in biopsies and lymphoid cell lines derived from this tumor secure the position of this carcinoma as an EBV-associated tumor.

The relationship between EBV and Hodgkin's disease (HD) is not as clear as that observed between EBV and BL or NPC. Clustering studies strongly suggest a horizontally transmissible agent as one factor in the pathogenesis of the disease. Although increased antibody levels to EBV have been recorded in persons with HD, it is clear that a significant percentage of patients have no detectable titers.

Although these arguments are highly suggestive, they are not conclusive as far as the etiology of BL and NPC are concerned. While there is good reason to believe that EBV is involved in the oncogenic process, its role could be that of an accessory factor or passenger virus in the tumor. No EBV DNA has yet been found in American BL tissues by the RNA-DNA hybridization technique, even though this tumor is histopathologically similar to African BL. RNA sequences homologous to that of the Rauscher murine leukemia virus RNA have recently been described in BL biopsies suggesting the possible interaction of Type C viruses in the genesis of this disease. If EBV acts to prime the cell for neoplastic change, it is possible that one or more combinations of environmental and host factors interact to promote oncogenesis. Thus, the association of EBV infection with more than one disease becomes more plausible. Should this virus prove to be a necessary co-factor in any or all diseases with which it appears to be associated, control of infection would be of paramount importance.

An expanding body of evidence has strengthened the causal relationship between herpes simplex virus type 2 (HSV-2) and carcinoma of the human uterine cervix, the second most common malignancy in women in the United States.

The frequency of antibody to HSV-2 is generally greater in women with cervical neoplasia than in normal controls matched for promiscuity and other risk factors.

All findings strengthen the association of HSV-2 with cervical carcinoma but still do not prove a causal relationship between the virus and this malignant disease. Studies to determine if this virus is the sole etiological agent, or an essential co-carcinogen in the induction of this disease will be continued.

3) Treatment and Control.

A rational approach to tumor therapy may not depend on the isolation of a bonafide human tumor virus. Indeed, existing model systems have provided sufficient information which can be applied to the control of human cancers. For example, chemical inhibitors (antienzymes, gene repressors) which act on specific stages of viral replication or stimulation or host immune mechanisms (vaccines) to virus or virus-mediated, tumor-specific antigens may prevent or control oncogenesis.

The systematic screening of rifamycin derivatives which block the RDDP activity of RNA tumor viruses may help in the development of inhibitors that possess properties for the control of specific gene expression. Some flouranthrene di-substituted cationic derivatives and analogs are also strong inhibitors of RDDP and of cell transformation.

Many compounds have been tested for their capacity to inhibit the reproduction of murine leukemia and sarcoma viruses in vitro. A few of the most active ones also showed inhibitory activity against these viruses in vivo. Among the chemicals tested for non-specific stimulation of the RES, imidazolethiazole, pyran copolymer, and Tilaron proved effective in increasing survival of mice in induced remission.

A DNA binding protein in non-virus producing AKR mouse cells has been isolated and found to have many of the properties of known repressor proteins described in bacterial virus systems. This finding could lead to understanding the mechanisms by which mammalian cells control gene expressions and how genetic mechanisms regulate RNA tumor virus expression.

Formalin killed vaccines prepared from murine leukemia viruses have produced significant reductions in tumor incidences in mice given potent chemical carcinogens. Because of the reported immunological and biochemical relatedness between MuLV (Rauscher) and human leukemic cells, terminal cancer patients were immunized with inactivated RLV. Evidence for humoral and/or cell-mediated immunity against this virus was found in more than one-half of the immunized patients. Whether this treatment produces beneficial effects for the patients can be only determined after many years of observation.

The immunogenicity of virus-induced tumor cells has been shown to be increased by infection with influenza virus. Tumor transplantation antigens on these cells can be isolated in cell-free form. This material protected mice against challenge with tumor transplants causing either suppression or complete regression. These studies have obvious implications for immunotherapy of human tumors.

SUMMARY

Between 30% to 90% of cancer deaths in man is preventable. Mortality statistics from around the world show that if cancer mortality in the United States, site for site was comparable with that of the lowest country reporting for each site, approximately 100,000 lives would be saved each year. The major sites in which gains should be made are cancer of the colon and the rectum, cancer of the lung, breast cancer, cervix and uterus (particularly among non-whites), cancer of the ovary, cancer of the prostate, and probably leukemia.

Information is already in hand to reach these goals for lung cancer and for cancer of the cervix and uterus. These 2 sites alone would account for 60,000 or more savable lives. A concerted research effort is called for in cancer of the lung, colon, breast cancer and cancer of the prostate. There is promise of substantial pay-off in these sites. For example, migrant studies have provided very strong evidence that cancer of the colon is a disease with a very strong environmental base, possibly related, among other, to high beef meat consumption. Japanese migrants to Hawaii show a 3 to 5-fold increase in contrast to Japanese on the home islands. When we find all elements of the new environment that lead to so large increases, application of this knowledge to the present United States population could reduce the U. S. incidence by another 25-30,000 lives a year.

Thirty percent (or 100,000 Lives) is a minimum estimate. If we add to the preventable cases (in terms of current knowledge, or knowledge that should be available in the next few years, earlier diagnosis and improved treatments--where prevention has broken down), this number should be easily doubled. For example, if the most recent knowledge on early diagnosis of breast cancer were applied across the country, we might anticipate 12,000 fewer deaths each year from breast cancer alone.

Current work on identifying the cancer "susceptibles", if successful, will lead to cutting down cancer deaths. First, by identifying susceptibles, we will know what people to watch closely and regularly, so that we might diagnose their disease early, and thus treat it successfully. Second, by identifying the cancer susceptibles, it should be possible to protect them from those environmental assaults which could be carcinogenic to them - while essentially innocuous to others.

Ninety percent reduction in cancer deaths could be reached within the present century, if, in addition to current knowledge on prevention, and universal application of the best diagnosis and treatment techniques, our research uncovered a) the interacting environmental elements that cause migrants' cancer rates to change toward those of their new countries; b) those environmental elements which lead to different rates in different countries in the world; and c) the susceptibles whom we must and can protect.

While there are major problems in the prevention of virus-induced cancers, not heretofore experienced in other areas of infectious disease, we should anticipate substantial inevitable success in the control (including prevention) of these diseases in man. These problems include the probable difficulties in using conventional vaccines for prevention if a major means of virus exposure is vertical rather than horizontal. Secondly, the "peaking" phenomenon of acute lymphocytic leukemia in Caucasian children at about 4 years of age and other similar phenomena suggest that it would take 5-10 years before the results of a preventative field trial were known. Even with a "clustering" disease of relative high incidence, such as Burkitt's lymphoma in equatorial Africa, nearly 1,000,000 children would have to be included in the "treated" cohort observed for 5-8 years to determine whether the expected 40-50 lymphomas would be eliminated or decreased.

These difficulties notwithstanding, opportunities for cancer prevention and control are very bright through application of existing knowledge and through a prioritied commitment to seek new information with innovative application for man.

A Note on Management. The obligatory opportunity to plan and manage biomedical activities from basic or fundamental research upwards through development and application, with effectiveness and efficiency, requires skillful staff and line personnel of unabashed motivation, objectivity, and tact. Tact is of paramount importance because NCI management must persuade constituent scientists to attack specific problems of human disease. We adhere to the belief that NIH exists primarily to allow people to live better and longer and that NCI abets this goal through the conduct of research and development for prevention and treatment of cancer in man. Tact, including the courage and reason to say no, is important also because while the need for widespread program participation is high, many scientists do not understand that even successful completion of their proposed project may not necessarily contribute to the attainment of program goals. This thrust towards the timely control of disease must be tempered with appreciation for and selection of fundamental projects with some expectation of contribution to eventual application to man. This can and should be done through both grants and contracts.

We are fortunate to have this admixture of talents and motivation in the Acting Deputy Director, Dr. G. B. Gori, and Associate Directors, Drs. Moloney, Saffiotti and Schneiderman; the Assistant to the Director, Dr. Depue, and in the Administrative Officers, Mr. John Miller and Mr. Nicholas Olimpio. Particular recognition is entirely warranted to their associates, whose motivation to program goals exceeds their desire for personal recognition.

Please refer to summary and individual reports for details of activities within DCCP programs for FY 1973.

CANCER ETIOLOGY AND PREVENTION PROGRAM

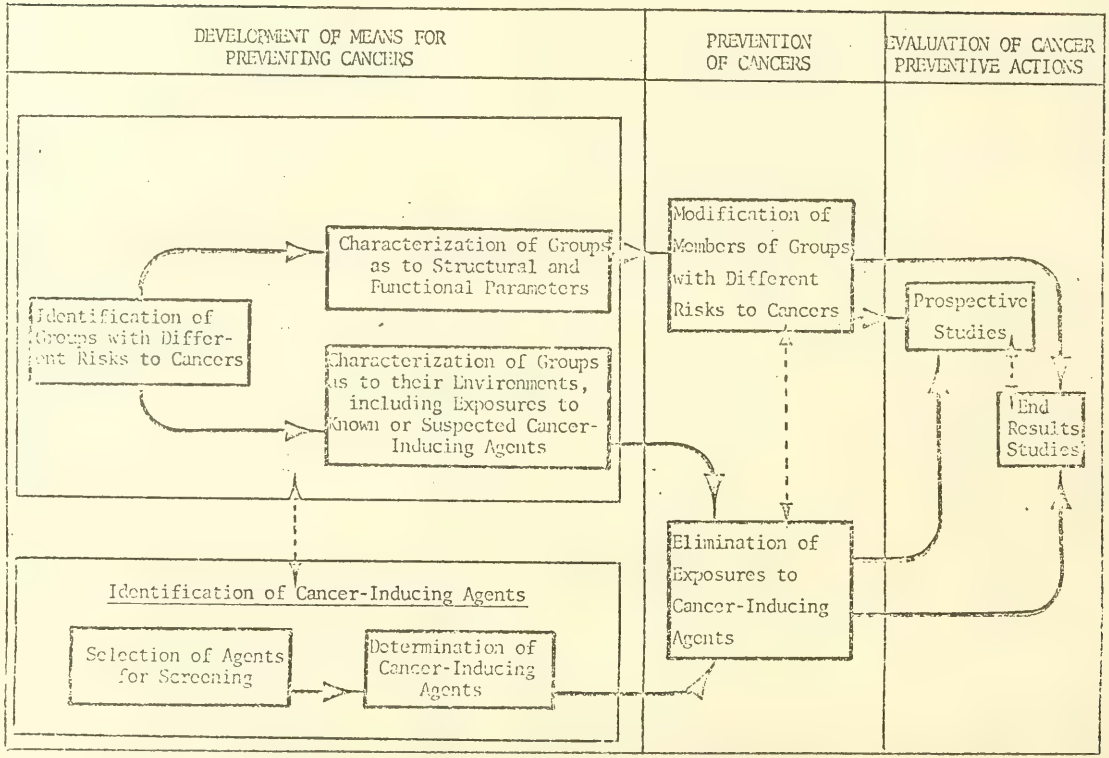
DEVELOPMENT OF MEANS FOR PREVENTING CANCERS
PREVENTION OF CANCERS
EVALUATION OF CANCER PREVENTIVE ACTIONS
Identification of Groups with Different Risks to Cancers
Characterization of Groups as to Structural and Functional Parameters
Characterization of Groups as to their Environments, including Exposures to Known or Suspected Cancer-Inducing Agents
Identification of Cancer-Inducing Agents
Selection of Agents for Screening
Determination of Cancer-Inducing Agents
Modification of Members of Groups with Different Risks to Cancers
Elimination of Exposures to Cancer-Inducing Agents
Prospective Studies
End Results Studies

FIGURE 1

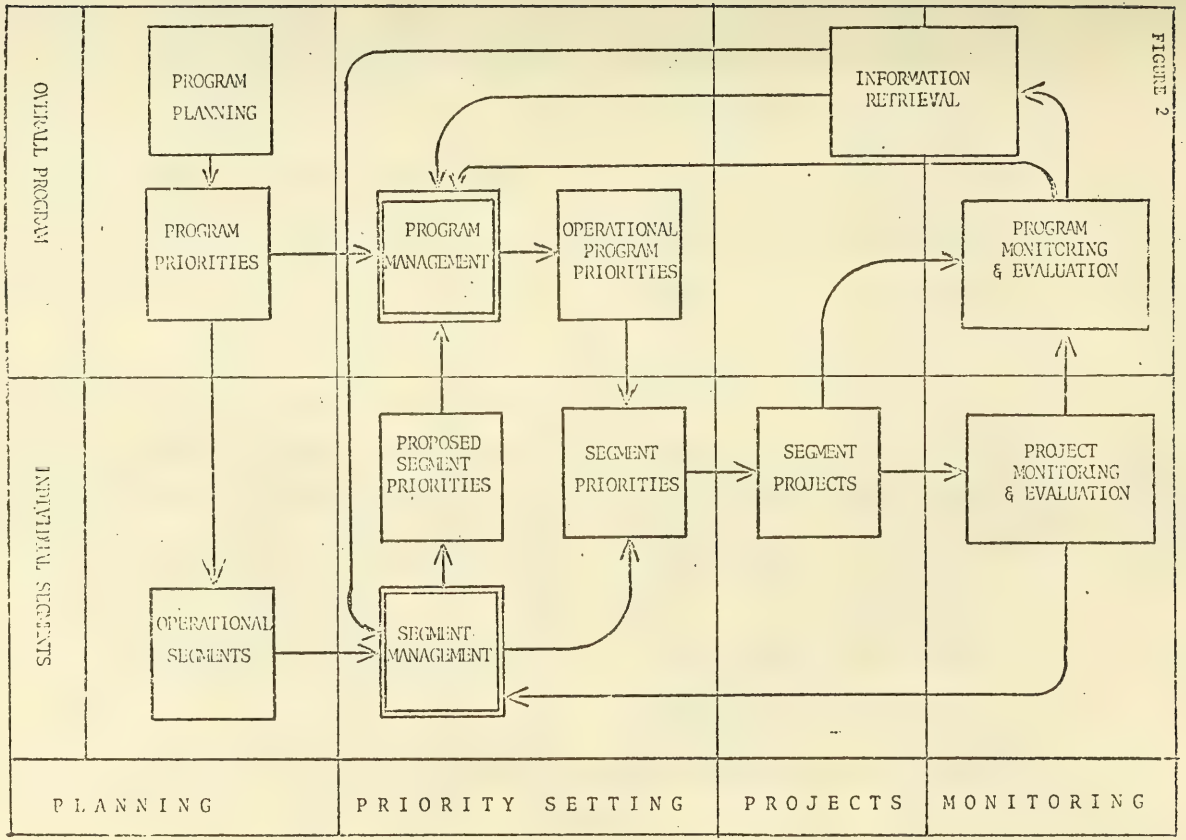
OVERALL PROGRAM
INDIVIDUAL SEGMENTS
PROGRAM PLANNING
PROGRAM PRIORITIES
OPERATIONAL SEGMENTS
PROGRAM MANAGEMENT
OPERATIONAL PROGRAM PRIORITIES
PROPOSED SEGMENT PRIORITIES
SEGMENT PRIORITIES
SEGMENT MANAGEMENT
SEGMENT PROJECTS
INFORMATION RETRIEVAL
PROGRAM MONITORING & EVALUATION
PROJECT MONITORING & EVALUATION
PLANNING
PRIORITY SETTING
PROJECTS
MONITORING

FIGURE 2

TABLE 1: CANCER INCIDENCE: EXPECTED NUMBERS, 1970-2000; CHANGE IN RATES, 1970-2000; DEATHS, 1970

Site	New Cases (Incidence)* 1970	2000	Deaths	Incidence Year 2000 % Change in Rate**	Major Causation	Means of Prevention
Lung	81,000	295,000	62,000	179	Tobacco Smoke Air Pollution (including on-the-job)	Stop smoking - Reduce pollution Less hazardous cigarette
Large and Small Bowel	92,000	134,000	44,000	11	Intestinal Flora? Heredity? Diet?	Viruses? Other insults. Identify susceptibles and eliminate their exposure.
Breast	82,000	111,000	30,000	4	Virus? Diet? (Hormones) Genetic?	Vaccines. Identify susceptibles.
Pancreas	20,000	35,000	18,000	38	Diet? Virus? Other Insults?	Identify etiology. Identify susceptibles.
Prostate	51,000	78,000	17,000	17	Hormones? Diet?	Identify etiology. Identify susceptibles.
Stomach	21,000	4,500	16,000	-84	Diet Poor socio-economic conditions	Diet modifications. Sociologic modifications?
Leukemias	20,000	32,000	15,000	26	Viruses. Radiation. Genetic.	Vaccines. Identify susceptibles and limit radiation.
Non-melanotic skin	376,000	585,000	5,000	20	Actinic rays. Genetic.	Limit radiation exposure. Identify susceptibles.
Misc.	242,000	408,000	75,000	30	Multiple	Identify extrinsic and intrinsic factors and modify them.

*Based on data from Third National Cancer Survey, 1969-1970. In situ cancers have been excluded.

**Projected change in age-adjusted incidence rates (year 2000 compared to 1970) assuming the trend in rates noted from 1947 to 1969 continues to the year 2000.

SUMMARY REPORT
MOLECULAR CONTROL WORKING GROUP
OFFICE OF THE DIRECTOR
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE
July 1, 1973 - June 30, 1974

The Molecular Control Working Group (MCWG) is a body of distinguished scientists which advises the National Cancer Institute on scientific findings in molecular biology which might offer potential leads to control or eradication of neoplastic processes in man. The objective of the MCWG is to apply the knowledge and techniques of molecular biology to the design of new drugs and other agents for the treatment of cancer and to the development of improved diagnostic and preventive methods for cancer.

The membership of the MCWG consists of:

Dr. T. E. O'Connor, Coordinator, Molecular Control, DCCP, NCI (Chairman)
Dr. C. Anfinsen, Chief, Lab. of Chemical Biology, NIAMDD
Dr. E. Borek, Dept. of Microbiology, Univ. of Colorado Medical Center
Dr. B. Chance, Professor, Biophysics & Physical Biochemistry, Johnson Research Foundation, Univ. of Pennsylvania
Dr. J. Fried, Professor, Dept. of Chemistry, Univ. of Chicago
Dr. R. Handschumacher, Professor of Pharmacology, Yale University School of Medicine, New Haven, Conn.
Dr. G. Gori, Deputy Director, DCCP, NCI
Dr. H. Jehle, Professor, University of Maryland
Dr. W. Joklik, Chairman, Dept. of Microbiology-Immunology, Duke University Medical Center, Durham, N.C.
Dr. S. Perry, Deputy Director, DCT, NCI
Dr. P. Stansly, Program Director for Cancer Biology, DCRR&C, NCI
Dr. J. Vinograd, Professor, Dept. of Chemistry-Biology, California Inst. of Technology

The activities of the MCWG are coordinated by the Coordinator, Molecular Control, who was until March 1974, administratively located in the immediate Office of the Director, DCCP, but who interacted with each of the Divisional Directors, NCI, in developing scientific aspects of the MCWG program pertinent to their Divisions. In March 1974, the Director, NCI, directed that an Operational Plan for the MCWG (see below) be implemented and the administration of the Molecular Control Program was transferred to the Division Cancer Research, Resources and Centers.

During the current reporting period, the MCWG convened on 6/8/73 and 12/14/73. The primary business at these open meetings was the formulation of an Operational Plan for the MCWG. At the 12/14/73 meeting, such a plan was unanimously recommended to the Director, NCI. The plan aims at the maximum coordination of the findings of molecular biology with a view to their speedy and effective application within the National Cancer Program, and provides mechanisms for the widest participation by the

molecular biology community in definition of existing research gaps and appropriate research goals. The plan envisions a Molecular Control Program which will comprise both Research Grants, aimed at development of the necessary basic research in the molecular biology of cancer, and Research Contracts aimed at providing the reagents and other necessary resources for execution of this research. Currently, an Administrative Plan is being formulated for implementation of the Molecular Control Program within the Division of Cancer Research, Resources and Centers.

RESEARCH PROGRAM

The research program of the MCWG, to date implemented under contract auspices, provided some notable successes during the current period:

(a) A screen for evaluating compounds for their antitumor or antiviral activity, which had been recommended by the MCWG, was supported by the Division of Cancer Treatment and implemented (March 1973) as Contract NO1-CM-33741 with Litton Bionetics, Inc., Bethesda, Md. The screen involves testing of toxicity of the compounds for mouse or human cells in culture and evaluation of their antiviral activity against infection of these cells with cancer viruses. In addition, the effect of the compounds on selected highly purified enzymes involved in virus and cell replication is evaluated.

In the first year of operation of this contract, primary efforts have been devoted to development and extensive modification of operating protocols. This phase of the work has been successfully concluded. The initial evaluation of the efficacy of this screen included a blind study of 43 compounds which included 18 known clinically effective antitumor drugs, as well as other antivirally effective and inactive compounds. The preliminary data indicate that 15 of the clinically effective antitumor drugs were identified as active materials by the screen. No false positives were recorded. The three failures comprise two alkylating agents which require liver activation *in vivo* and one antitumor antibiotic. All the known antiviral compounds (e.g. Streptovaracins) were detected. These findings support the dual capacity of the screen to detect either antiviral or antitumor activity. The technical basis for this is that formation of foci of morphologically transformed cells in mammalian cell cultures by sarcoma viruses requires both continued cell division and spread of progeny virus to surrounding cells. Thus, focus inhibition can reflect specific inhibition of virus replication and/or toxic inhibition of transformed cell growth. Examination of the toxicity of the compounds for uninfected and transformed cells, as determined by cell growth kinetics and uptake of labelled precursors in DNA, RNA and protein, and determination of the effect of the compound on virus yield, can provide insight into the *modus operandi* of the compounds. Such studies are now underway on the known clinically effective drugs. It is also planned to use the screen in examination of combinations of known drugs, in quantitative comparison of analogs of known drugs, and in study of reversal of toxicity of known drugs. Such studies could provide a highly useful adjunct in the design of drug regimens for *in vivo* animal and human clinical trials.

Use of the screen confirmed earlier results by other workers (Green, Gallo, Calvin) that various synthetically modified Rifamycin antibiotics could inhibit the activity of the reverse transcriptase enzyme of oncorna viruses and in some instances prevent focus formation by sarcoma viruses in cell cultures. However, it was observed that some of the parent Rifamycin antibiotics - Rifamycin B, Rifamycin O, Rifamycin S and Rifamycin SV - inhibited focus formation by murine sarcoma virus on mouse cells and feline sarcoma virus on human cells even though they did not inhibit the enzymatic activity of the viral polymerase. Rifamycin S was shown to produce a several hundred fold diminution in virus yield at concentrations of 4µg/ml and to exert this effect through inhibition of an early step in virus infection following virus penetration, rather than by a selective toxicity for either the uninfected or transformed cells. This new finding of antiviral activity in parent Rifamycins (secreted by Streptomyces mediterranei) led to a reappraisal of previously reported antiviral activity by other antibacterial antibiotics. In the present screen some purified streptovaracins (secreted by Streptomyces spectabilis) also inhibit oncorna virus replication without pronounced inhibition of oncorna virus polymerase activity. Streptonigrin (secreted by Streptomyces flocculus) can inhibit transformation of human cells by feline sarcoma virus at concentrations as low as 0.1 ng/ml, but this effect appears to arise from an arrest of cell division. Thus, the antiviral activities of the various antibiotics, like the antibacterial activities, appear to arise from various mechanisms. The most potent compound examined to date is Maytansine (70% inhibition of focus formation on mouse 3T3 cells @ 0.05 ng/ml). Antibiotics which interfere with oncorna virus replication (eg. Rifamycin S) would appear to merit examination of their capacity to interfere with other non-cancer viruses.

The Contract Narrative for this contract is included with other Contract Narratives of Drug Research and Development, Division of Cancer Treatment, which funded and administered this contract. The Coordinator for Molecular Control acted as Project Officer.

(b) Under Contract NOI-CP-3-3231 with the General Electric Company, Schenectady, N.Y., collaborative studies between G.E. investigators and investigators within the National Cancer Program on the application of advanced optical and electrical techniques to problems in oncology were continued. The G.E. investigators in collaboration with investigators at the John L. Smith Cancer Memorial laboratory of the Charles Pfizer Co. constructed and evaluated an electrical resistance pulse analyser for detecting and counting virus particles. The prototype instrument readily detected and counted various oncorna viruses at concentrations of $\sim 10^8$ virions/ml in times of about 1 minute. This instrument has now been placed in operation at Maywood for evaluation in routine monitoring of production of various oncorna viruses. The cost of this prototype construction was $9,600. A more versatile research instrument has also been constructed and is currently being evaluated for detection of virus - antibody complexes and as a detector in antiserum neutralization titrations of viruses. This instrumentation could vastly facilitate diagnostic virology and virus production monitoring and has generated enquiries from pharmaceutical companies.

Two techniques involving thin film technologies are proving useful in study of proteins and glycoproteins and may provide alternatives to the established radioimmune assay in immunology. Dr. Ivar Giaever, who shared the 1973 award of the Nobel Prize in Physics, has applied his method of specific adsorption of proteins to monomolecular protein layers to the detection of carcinoembryonic antigen in collaborative studies with Dr. Zamcheck at Harvard Medical School and Dr. T. Borsos and Dr. H. Rapp, NCI. In this method the antigen (or antibody) is coated onto a pristine metal surface to form a monolayer. Exposure to solutions of the corresponding specific antibody (or antigen) then produces a double protein layer which can be detected visibly - or more quantitatively by a light polariscope. This technique has considerable diagnostic potential, but its use in CEA studies requires further evaluation. The second technique, Alternating Streaming Current Analysis (ASCA), involves measurement of electric charges generated by electrolyte oscillating over protein membranes (or protein adsorbed as monolayer). This method appears promising for detection of protein conformational changes and for detection of specific antigen-antibody interactions.

(c) Under Contract NO-CP-33226 with the Michigan Cancer Foundation, Detroit, Mich. (with a subcontract to the Scientific Laboratories, Ford Motor Company) various aspects of laser spectroscopy were applied to problems in oncology and cancer virology. Laser beat frequency spectroscopy was found to be a useful method for detecting and quantitating oncorna viruses, although of perhaps less general utility than the G.E. resistance pulse counter. A finding of more general importance was the observation that the laser Raman spectra of DNA observed during thermal denaturation reflect a "premelting zone" as well as the hyperchromicity observed both in Raman spectra and UV absorption spectra during true melting. It is speculated that this "premelting zone" involves semi-dissociation of poorly matching nucleotide sequences and hydrogen bond dissociations, whereas the true melting involves nucleotide base unstacking. Since the laser Raman spectra of each of the four bases has distinctive frequencies, computer recording and analysis of spectra of DNA during thermal dissociation and reannealing may provide important clues to the structural organization of DNA. Currently, the structural characteristics of viral, prokaryotic and eukaryotic DNA are being explored with this technique.

OVERVIEW OF MOLECULAR CONTROL PROGRAM

A truly national cancer program necessarily involves the participation of the most appropriate available advanced technology in the solution of relevant research problems. However, the technological strength of the U.S.A. does not lie solely in the content of the available technology, but also includes the creative interaction of the components of our society - industry, academia, research institutes and government - in developing novel approaches to challenging problems. The contract (NO1-CP-3-3231) with the General Electric Company for the application of advanced optical and electric technology to problems of the National Cancer Program, involving as it does collaboration with another industrial firm (Charles Pfizer, Co.), Harvard Medical School, and NCI, may be viewed in

this context. Likewise, the contract with Michigan Cancer Foundation (NO-CP-33226) involves government support for a regional center for scientific studies in cancer (Michigan Cancer Foundation at Detroit) with a local source of outstanding industrial technology (the Ford Motor Company Research Laboratories at Dearborn, Mich.). The critical feature in such interactions is the matching of the physical and industrial technology to truly pertinent and fundamental biological problems. The development and maintenance of such relevance appears best attainable by constant dialog between the participants from each component. During the current period, scientists of NCI and GE have visited and worked in each others' laboratories as have scientists of the GE and Charles Pfizer Company.

The large number of technical proposals received by the MCWG suggests that the above examples constitute only initial steps in the optimal involvement of available technology in an interdisciplinary way under the Molecular Control Program. It appears desirable that this growth be channelled in an orderly way by organization around major unsolved scientific problems of critical relevance to the National Cancer Program. Dr. Britton Chance (member, MCWG) presented this viewpoint to the National Cancer Board at a meeting in November 1973 and suggested that a multidisciplinary scientific probe of the structure and function of the membranes of normal and malignant cells was necessary and could yield basic information for subsequent rational design of cancer control methods. It is hoped that an interdisciplinary body of participating scientists will be organized to undertake this task during the coming year.

The above emphasis on the hitherto somewhat underdeveloped contributions of biophysics should not be interpreted as defining the Molecular Control Program. This new technology will ultimately have to be appraised in terms of its contribution to better understanding of carcinogenesis, development of improved diagnostic techniques and provision of techniques for elucidation of vital biological mechanisms. The new screen (Contract NO1-CM-33741) for antiviral and antitumor compounds should vastly facilitate the task of the molecular biologist in the rational design of antitumor drugs and should potently augment the other approaches already supported by the Division of Cancer Treatment. The full cooperation of staff members of the Division of Cancer Treatment, particularly that of Dr. S. Schepartz, Dr. H. Wood, and Ms. N. Lomax, was vital in the launching of this new program.

During the coming year, the Operational Plan for the Molecular Control Program calls for the participation of members of the molecular biological research community in converging attacks on research targets, deemed by the MCWG as vital to rational approaches to solution of the cancer problem. This will require a major leadership role by eminent scientists outside the government community. It is anticipated that this new endeavor will be supported by a mix of Grant and Contract funding.

An interdisciplinary approach to a problem can run the danger of unnecessarily duplicating existing programs or intruding on the orderly

administration of vital existing programs. Conversely, the time spent in demarcation of such dangers can be extended until the climate for solution of the technical problem is dissipated, particularly when the free and enthusiastic involvement of scientists is vital to the solution. The Molecular Control Program has received indications of support from the molecular biology community. It also has received support from sectors of the NCI community. It to date has produced tangible scientific research results. The administrative structuring of this program and its interaction with other programs within NCI has been subject to extensive examination. Hopefully, these administrative problems have finally been resolved and the Molecular Control Program can proceed with its task of coordinating and applying molecular biology to the benefit of the cancer patient.

RESEARCH PERFORMED ON OTHER NCI PROJECTS

Objectives:

The isolation and characterization of viruses from human tumor materials and characterization of their possible etiological role in human neoplasia.

Methods Employed:

Propagation and characterization of viruses in tissue cultures and characterization of these viruses by immunological and nucleic acid hybridization procedures.

Major Findings:

In the last annual report, the chemical induction of an oncorna virus in low yield from a tissue culture cell line (SA4-SO) originally established from a human liposarcoma was described. During the current period, the cell line was supplied to Frederick Cancer Research Center where, in collaborative studies with Dr. G. Shibley and his associates, it has been propagated in monolayer, roller bottle and suspension cell culture. In addition, various clones of this cell culture have been established by Dr. Roman Pienta and propagated to mass culture. Dr. Nelson Reese and associates (Naval Research Biological Laboratory) have studied the karyotype and isozyme characteristics of various passages and clones of the SA4-SO cultues. All cells examined have been found to be of human karyotype and to contain a translocated γ chromosome and to have an isozyme pattern consistent with that of the original black patient donor. No PPLO has been detected in repeated tests.

The virus is inducible by hormonal and/or chemical induction. In early runs the virus was strongly cell associated, but improvements in induction procedures have recently yielded cell-free virus which can be harvested from the culture fluids. Preliminary data indicate that certain cell clones sporadically shed virus spontaneously. The difficulty in definitively characterizing this virus to date has been the low but consistently improving yields of virus. Immunological tests indicate that the virus serologically differs from the Mason Pfizer virus. The virion

has been visualized as an oncorna virus by critical point drying and electron microscopic techniques and has been shown to contain a "reverse transcriptase" enzyme and a 70S RNA by the simultaneous technique and to have a buoyant density of 1.16 g/cm on sucrose gradients. The reverse transcriptase has been purified to homogeneity and characterized as a 70,000 MW protein by gel electrophoresis and shown to serologically partially react with Wooly Monkey Sarcoma Virus reverse transcriptase, but not with a similar enzyme isolated from mouse leukemia viruses. Preliminary experiments indicate that the virus can be propagated in other human, primate or bovine cells. Current emphasis is on preparation of sufficient quantity of DNA transcript probe to permit execution of definitive experiments on homology of the genome of this virus with unique nucleotide sequences present in cells of human sarcomas and determination of species of origin of this virus.

Significance to Biomedical Research and the Program of the Institute:

Definitive characterization of an oncorna virus as of human origin and as having a genome containing nucleotide sequences homologous to unique sequences present in the DNA of human neoplasias should provide a viral reagent for the study of the chemotherapeutic and immunological control of viral-associated human cancers.

Proposed Course:

Studies on the species of origin and the possible etiological role of the above virus will be pursued to a definitive conclusion.

Honors and Awards:

Recipient of President's Award, Leukemia Society of America (1974)

PUBLICATIONS OF DR. TIMOTHY E. O'CONNOR

1. Chan, E.W., Schiop-Stansly, P.E. and O'Connor, T.E. "Mammalian Sarcoma Leukamia Viruses 1: Infection of Feline, Bovine and Human Cell Cultures with ST-FSV." J. Nat. Cancer Inst. 52 (February, 1974)

2. Chan, E.W. Schiop-Stansly, P. and O'Connor, T.E. "Rescue of a Cell-Transforming Virus from a Non-Virus Producing Bovine Cell Culture Transformed by a Feline Sarcoma Virus." J. Nat. Cancer Inst. 52 (February, 1974)

3. O'Connor, T.E. "Molecular Control Program." Cancer Chemotherapy Reports, Part 3, Vol. 4 (1973).

4. O'Connor, T.E. "Reverse Transcriptase - Progress, Problems and Prospects." In: Unifying Concepts of Leukemia Bibl. Haemat. No. 39, R.M. Dutcher and L. Chieco-Bianchi (Eds.) pp. 1165-1181 (Karger/Basel) (1973)

5. O'Connor, T.E. "Summation." Seventh Annual ASCP Res. Symp. on Molecular Mechanisms in Viral Neoplasia of Animals and Their Implication in the Origin of Cancer in Man. (W.O. Russell, Chairman) Amer. J. Clinical Path. 60: 100-108 (1973)

6. O'Connor, T.E., Schiop-Stansly, P., Sethi, V.S., Hadidi, A., and Okano, P. "Antibiotic Control of Infection of Human or Mouse Cells with Oncorna Viruses." In: Pharmacol. Basis of Cancer Chemotherapy, Proc. 27th Ann. Symp. on Fundamental Cancer Res., M.D. Anderson Hospital and Tumor Inst. (1974 - In Press)

7. Rauscher, F.J. and O'Connor, T.E. "Virology" In: Cancer Medicine, Holland, J.F. and Frei, E. (Eds.) Lea and Febiger, Phil. pp. 15-44 and pp 1931-1932 (1973)

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MOLECULAR CONTROL PROGRAM

July 1, 1973 - April 1, 1974

Part A - Published Papers

Author Index

DeBlois, R.W./ 1
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O'Connor, T.E./ 3
Schidlovsky, G./ 1
Uzgiris, E.E./ 4
Wolff, J./ 1

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Hadidi, A./ 3
Kaplan, J.H./ 4
Liebes, L./ 1
McCormick, J./ 1, 2
O'Connor, T.E./ 3
Okano, P./ 3
Rich, M./ 1
Rimai, L./ 1
Salmeen, I./ 1
Schiop-Stansly, P./ 3
Sethi, V.S./ 3
Uzgiris, E./ 4

A. PUBLISHED PAPERS

1. DeBlois, R.W., Wolff, J.S. and Schidlovsky, G. "Viral Analysis by Resistive Pulse Technique." Biophys. J. 13: 303a (1973)

2. Giaever, I. "The Antibody-Antigen Reaction: A Visual Observation." J. Immunol. 110: 1424 (1973)

3. O'Connor, T.E. "Molecular Control Program." Cancer Chemo. Reports, Part 3, Vol. 4 (1973)

4. Uzgiris, E.E. and Kaplan, J.H. "Protein Coated Electrodes." Rev. Sci. Instruments, Jan. 1974

B. PAPERS IN PRESS

1. Gill, D., Rimai, L., Salmeen, I., Liebes, L., Rich, M., McCormick, J. "Electrophoretic Light Scattering (ELS) of Rauscher Murine Leukemia Virus." Biophys. J. (In Press)

2. McCormick, J. "Physical Separation of Nuclei from Two Independent Plasmodia of Physarum Polycephalum Following Fusion." J. Cell Biol., (In Press)

3. O'Connor, T.E., Schiop-Stansly, P., Sethi, V.S., Hadidi, A., and Okano, P. "Antibiotic Control of Infection of Human or Mouse Cells with Oncorna Viruses." In: Pharmacological Basis of Cancer Chemotherapy, Proc. 27th Ann. Symp. on Fundamental Cancer Res. of the M.D. Anderson Hospital and Tumor Institute. (In Press)

4. Uzgiris, E.E., Kaplan, J.H. "Study of Lymphocyte and Erythrocyte Electrophoretic Mobility by Laser Doppler Spectroscopy." Biochem. Biophys. Res. Comm. (In Press)

GENERAL ELECTRIC COMPANY, SCHENECTADY, N.Y. (NO1-CP-3-3231)

Title: Collaborative Efforts with Investigators in the National Cancer Program for Application of Advanced Optical and Electrical Technology to Oncology

Contractor's Project Director: Dr. Milan Fiske

Project Officer (NCI): Dr. Anthony Kontaratos

Objectives: The application by collaborative studies of advanced optical and electrical techniques to critical biological problems arising within the National Cancer Program.

Methods Employed: Laser beat frequency spectroscopy, laser Doppler spectroscopy, electrical resistance pulse analysis, alternating streaming current analysis, protein film monolayer and bilayer adherence to pristine metal surfaces measured by light polarization.

Major Findings: A prototype instrument for virus counting, based on electrical resistance pulse analysis, was constructed and evaluated in collaborative studies with investigators at the John L. Smith Memorial Laboratory of the Chas. Pfizer Co. at Maywood, N.J. The instrument was found capable of counting Rauscher leukemia virus and other oncorna viruses at concentrations of 10^8 virions/ml in times of ~ 1 minute and lower virion concentrations at correspondingly longer times. Thus, the instrument appears useful for monitoring virus production, and evaluation of its use for routine quality control of virus production is continuing. A more versatile research instrument has been constructed. Preliminary data indicate that this instrument can detect virus-antibody complexes and has potential, if some mechanical problems can be overcome, of use in rapid serological titrations of virus. Technical problems in Doppler spectroscopy including bubbling and decompositions at electrode surfaces have been overcome and the electrophoretic mobility of human lymphocytes has been determined. Carcinoembryonic antigen has been detected with a sensitivity comparable to that attainable by the radioimmune assay by interaction of specific antibody with antigen adsorbed as a protein monolayer on pristine metal surfaces and as detected visibly or by polariscopy.

Significance to NCI Programs and Biomedical Research: The development of the resistance-pulse virus counter provides a relatively cheap instrument (prototype instrument costs $9,600) which permits rapid detection and counting of cancer viruses, and virus-antibody complexes. This development facilitates quality control of virus production and diagnostic virology. The detection of carcinoembryonic antigen by interaction of protein thin films appears to provide a simple sensitive diagnostic tool to immunologists

which can be applied in examination of other antigen-antibody systems relevant to diagnostic oncology.

Proposed Course of Project: This project will be continued. Emphasis will be placed on application of the resistance pulse analyzer in diagnostic virology and the interaction of protein monolayers in diagnostic immunology.

Date Initiated: 1/2/73

Current Annual Level: $310,700

MICHIGAN CANCER FOUNDATION, DETROIT, MICH. (NOI-CP-33226)

Title: Inelastic Laser Light Scattering Studies on Nucleic Acids, Nucleoproteins and Viruses

Contractor's Project Director: Dr. J. J. McCormick

Project Officer (NCI): Dr. T. O'Connor

Objectives: Application of laser light spectroscopy to characterization of viruses, virus antibody complexes and nucleic acids.

Major Findings: Laser beat frequency spectroscopy was used to measure diffusion constants, and by inference hydrated diameters, of several strains of mouse leukemia virus as harvested from infected tissue cultures. Protocols were developed for detection and quantitation of virus in body fluids such as milk and plasma. The thermal denaturation and renaturation of bacterial and mammalian cell DNAs were studied. In such denaturations the laser spectra revealed a preliminary dissociation or "premelting zone" not revealed by conventional ultraviolet hyperchromicity shifts.

Significance to Biomedical Research and the Program of the Institute: Laser spectroscopy appears to be a useful tool for characterization of cancer viruses prepared from complex body fluids. Laser spectroscopy is giving new insight into the thermal denaturation and renaturation patterns of DNA isolated from various sources and may provide understanding of structural features of these genetic molecules.

Proposed Course: This project will be continued with emphasis on laser spectroscopy characterization of viruses associated with mouse or human mammary tumors as obtained from milks. Preliminary observations on the quantitation of oncorna viruses by fluoresence with laser light of viral nucleic acid-acridine orange complexes will be followed up with the hope of providing a simple virus quantitation procedure. Laser spectra of DNA measured during thermal denaturation and reassociation will be examined as a tool in elucidation of animal cell DNA structure.

Date Contract Initiated: 1/2/73

Current Annual Level: $251,634

Annual Report

Smoking and Health Program
Division of Cancer Cause and Prevention
National Cancer Institute

July 1, 1973 through June 30, 1974

Introduction

Since the 1964 Report of the Surgeon General and later studies, it is less and less disputed that tobacco smoking increases dramatically the lung cancer risk in man. By now, it is well established that cigarette smokers have a higher risk as compared with nonsmokers, and a direct connection has been found between cumulative dose of exposure and risk, as well as a reduction of risk following cessation of smoking. Also, the types of cancer associated with cigarette smoking are different, when compared with other cancers of the same tissue in nonsmokers. Moreover, similar correlations exist for diseases other than cancer, such as cardiovascular and respiratory ones.

It is probable that if tobacco smoking were to be proposed at this time in history, it would be prohibited as a most dangerous public health hazard. But things being what they are, tobacco is and has been for over three centuries the economic backbone of several States of the Union and has attained an unprecedented status in the mythology of our culture. It is also a conspicuous source of tax revenue for the federal and local governments. Today it appears unworkable to propose the immediate abolition of tobacco smoking, or to expect that campaigns in this direction may attain success in a short time; it is a realistic conclusion that people will continue to smoke in large numbers.

The use of tobacco poses a dual demand for public health action: to promote public awareness of smoking hazards, and to reduce the risk of those who continue to smoke, by devising protective and preventive measures.

Public education campaigns have been effectively sponsored by the National Clearinghouse for Smoking and Health, now part of the Center for Disease Control, DHEW, and by the American Cancer Society, the American Health Association and other private organizations with public health interests.

On the other side, the coordinated efforts of the National Cancer Institute's Smoking and Health Program have included the National Heart and Lung Institute and the United States Department of Agriculture, and are specifically directed at the identification of individuals most likely to develop smoking related cancers and diseases, to means of reducing their risk, and to the development of cigarettes less hazardous than those available today.

The first efforts at the Institute were directed at collecting the most qualified information and advice on the nature of the hazard and opportunities for its abatement. The Tobacco Working Group was established over six years ago as a branch of the Lung Cancer Task Force. This group of eminent scientists represents affiliations ranging from the tobacco industry to the academic world, private research organizations and foundations, to the United States Department of Agriculture, the National Institutes of Health, National Environmental Health Sciences, National Heart and Lung Institute, and the National Cancer Institute. Their qualifications cover the entire range of specialities in the field: agronomy and tobacco genetics, tobacco curing, cigarette manufacturing, tobacco and smoke chemistry bioassay of smoke and smoke condensate, pathology and epidemiology of smoking in man. The reality of the smoking hazards and the problems they pose have been acting as powerful unifying elements for the often divergent interests concerned, and the Tobacco Working Group has been unusually successful in the definition of areas of interest and in providing operational guidelines and standardized methodologies.

Markers of individuals at higher risk may include inborn characteristics and environmental factors such as genetic defects, physiologic and pathologic concurrences, nicotine dependency, motivation, occupation, social habits, smoke intake and smoking mechanics, etc. It is necessary to develop methods for the identification of these markers. Also, pharmacologic and clinical methodologies must be devised to help the high-risk smoker who should but finds it difficult to quit; these include the study of drugs that aid in smoke cessation or can counteract specific deleterious effects of smoking, and physiologic and motivational research in clinical approaches to smoke cessation. Retrospective and prospective studies showing a dose-response relationship indicate that a reduction of exposure to tobacco smoke should result in reduced incidence of smoking related cancers and diseases. In fact, during the past 20 years a dramatic reduction in the amount of tar and nicotine yields of American commercial cigarettes has taken place; recent epidemiological studies suggest that a smoker today must smoke more of modern cigarettes than he would have of cigarettes available 20 years ago, in order to develop the same risk of disease, and that smoking filter cigarettes for 10 years or more significantly decreases the risk of lung cancer as compared to smokers of unfiltered cigarettes. All this points to the desirability of filter cigarettes and other low yield products on the market.

Moreover cigarettes can be engineered to produce smoke which, on an equal dose basis, can produce higher or lower carcinogenic response and other pathologic manifestations in animals. Choice and manipulation of appropriate tobacco types, engineering of the cigarette and selective filtration will permit removal of a considerable portion of pathogenic smoke components, and still maintain consumer acceptability. This should result in smoking products which are intrinsically less injurious to human health, over and above the simple reduction of tar and nicotine.

The improvement of some cigarette characteristics may possibly result in worsening others; close collaboration of experts in carcinogenesis, cardiovascular and respiratory diseases and tobacco technology is mandatory to insure that cigarettes become less hazardous for as many as possible of their potential effects in man. The National Cancer Institute also coordinates with the National Heart and Lung Institute and other public health agencies in pharmacologic research and clinical application of motivation studies leading to smoke withdrawal techniques for the high-risk smoker. A general plan of research approaches is shown in Chart I.

Scientific Review

The NCI Smoking and Health Program is subject to a three-tier review process which proceeds from broad policy and strategic considerations to specific establishment of project priorities, to detailed technical evaluations. Throughout the process, the Director of the Smoking and Health Program has the responsibility to guide and direct the program utilizing the established advisory mechanisms in the ways most effective to meet program requirements.

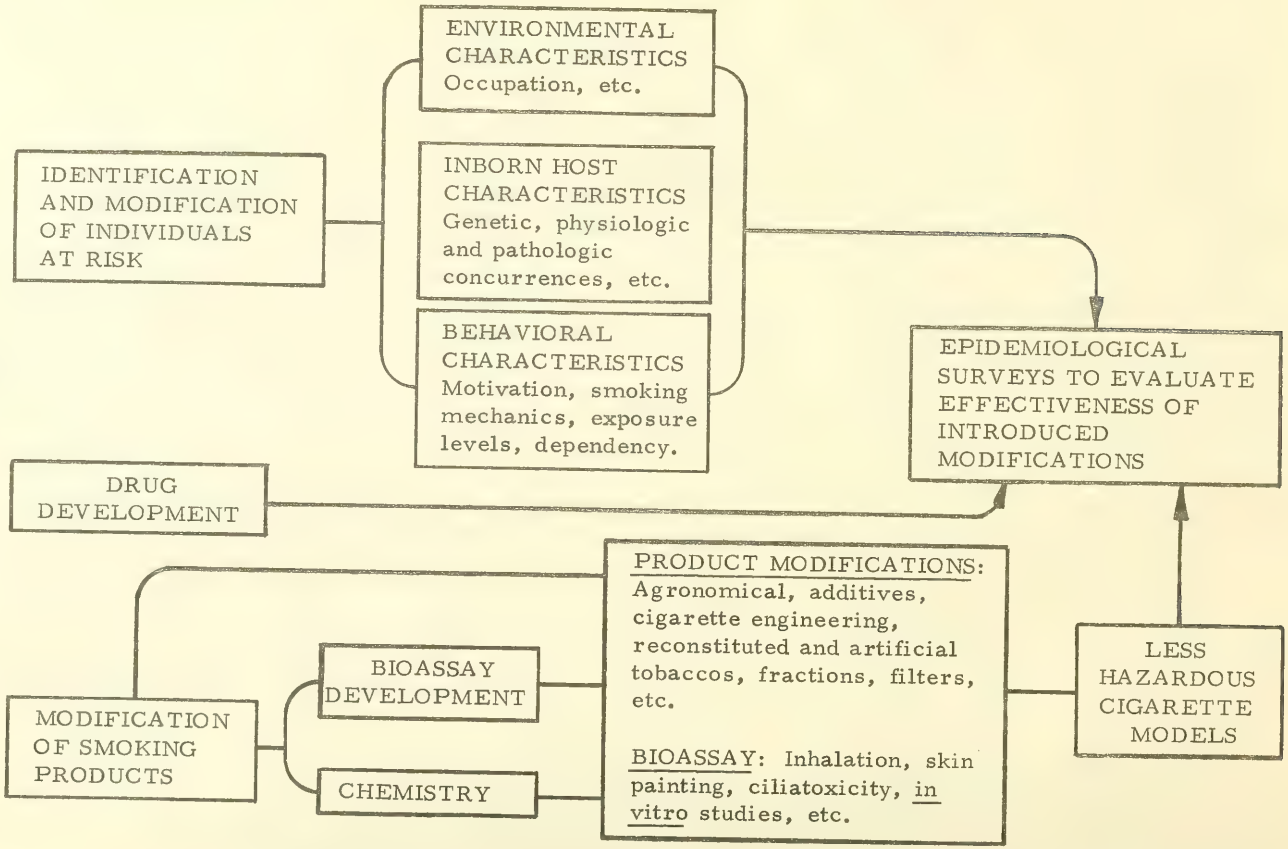
- The first level of review is concerned with overall program strategy, objectives, and major lines of investigation. The Tobacco Working Group has been established to provide relevant information and guidance from the most qualified representatives of the governmental, industrial, institutional and academic sectors.

- The second level of review deals with individual projects, and its specific purpose is to establish the need, the relevance and the relative priority of each project. This function is carried out by the Smoking and Health Review Board, a group of government specialists assisted by outside consultants on an ad hoc basis where required.

- The third level of review is to provide detailed technical evaluation of individual projects (contracts). Because the Smoking and Health Program requires diversified array of scientific disciplines (e.g. cardiology, pathology, hematology, oncology, biochemistry, aerosol and filtration physics, physiology, engineering, analytical chemistry, plant genetics, agronomy, and numerous specialized fields of technology), it is impossible to form a standing committee of reasonable size to perform technical evaluation of all projects. Accordingly, the Director, Smoking and Health Program, utilizes the ad hoc efforts of eminent specialists to accomplish individual technical evaluations as required.

CHART I

RESEARCH PLAN TO DECREASE THE RISK OF SMOKING RELATED CANCERS AND DISEASES


ENVIRONMENTAL CHARACTERISTICS Occupation, etc.
IDENTIFICATION AND MODIFICATION OF INDIVIDUALS AT RISK
INBORN HOST CHARACTERISTICS Genetic, physiologic and pathologic concurrences, etc.
BEHAVIORAL CHARACTERISTICS Motivation, smoking mechanics, exposure levels, dependency.
EPIDEMIOLOGICAL SURVEYS TO EVALUATE EFFECTIVENESS OF INTRODUCED MODIFICATIONS
DRUG DEVELOPMENT
PRODUCT MODIFICATIONS: Agronomical, additives, cigarette engineering, reconstituted and artificial tobaccos, fractions, filters, etc.
BIOASSAY: Inhalation, skin painting, ciliatoxicity, in vitro studies, etc.
BIOASSAY DEVELOPMENT
CHEMISTRY
MODIFICATION OF SMOKING PRODUCTS
LESS HAZARDOUS CIGARETTE MODELS

Lists of Tobacco Working Group Members, Smoking and Health Review Board Members and consultants follow.

Tobacco Working Group

Dr. Gio B. Gori (Chairman)
Acting Deputy Director
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National Cancer Institute

Dr. William W. Bates
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Liggett & Myers Incorporated

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Dr. Peter Hill
American Health Foundation

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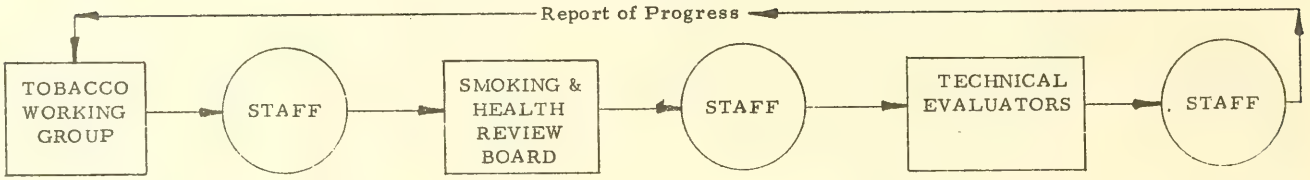
Arthur D. Little, Inc.

Dr. Ernst Wynder
President, American Health
Foundation

FLOW DIAGRAM 1

RELATIONSHIP OF ACTIVITIES IN THE
SMOKING AND HEALTH PROGRAM



Advise on Objectives and Programs.	Establish Projects. Write RFPs and Changes to Existing Projects. Prepare Project Plans.	Recommend Relevance and Priority of Projects.	Request Proposals. Prepare Review Review Summary Sheets.	Select Best Proposals. Evaluate Experimental Protocols.	Implement and Monitor Projects. Report to TWG on Progress and Problems.

Achievements - Summary

Starting in 1968 the Tobacco Working Group set out to identify the criteria and methods for development of less hazardous smoking products. Agronomical and industrial practices of cigarette manufacturing were examined and a consensus was reached on optimum methods for chemical analysis of tobacco smoke components, and for the design of biological testing methods in laboratory animals. Criteria to determine the smoking hazard in relation to cardiovascular and respiratory diseases were also studied. The Tobacco Working Group recommended experimental designs for testing several cigarette types.

The results of the first set of experiments initiated in 1970 have been evaluated. They indicate that by increasing the porosity of the cigarette paper the condensate resulting from the cigarette smoke is less carcinogenic when painted on mouse skin. The results also indicated that processed tobaccos known as reconstituted tobacco sheets, can be manipulated to yield condensates of lower carcinogenic activity for mouse skin than the condensates of the original tobacco. The results also give preliminary indication that some processing methods for reconstituted tobacco sheets are better than others, and on this basis a new set of experiments have been designed to further clarify the influence of manufacturing processes and therefore to select the most desirable ones.

Analysis of the second set of experiments begun in 1971 indicates that man-made smoking materials can be manufactured to yield condensates of very low carcinogenic activity for mouse skin.

Also, the first set of experiments appears to indicate that certain lipid soluble fractions of tobacco may contain specific precursors of undesirable smoke components. A more precise analysis of these components and their precursors is now underway, to allow their selective removal from tobacco.

Results indicate that carcinogenicity of marijuana smoke condensate is comparable to that of tobacco smoke, and that it contains similar chemical components, with the exception of cannabinols which are present instead of nicotine.

Development of less hazardous cigarettes is conducted primarily by a series of large collaborative experiments in which experimental cigarettes are evaluated for carcinogenicity and other hazards, controlling variables are identified in the cigarette and smoke from it, and new models are designed for further test. These experiments each involve about 25 to 30 experimental and reference cigarette types, which are machine smoked to form condensate which is painted on the dorsal skin of about 200 mice per cigarette type (plus controls) for 18 months. Analyses are made of many chemical and physical parameters in the tobacco blends, cigarette characteristics, gas and particulate phases of smoke, and condensate. Correlations are sought between these analyses and the relative tumorigenic potency of the condensates, so that new experimental variables can be formulated.

The results are consistent and rational, and have already led to specifically designed less hazardous cigarettes for bioassay. Factors showing strong positive correlation with tumorigenicity in the first experimental set of cigarettes include nicotine and waxes (hexane solubles); these may account for the good performance of reconstituted tobacco sheets made by processes which reduce these and other objectionable constituents. Inert tobacco diluents appear to be favorable; a similar effect may occur in "expanded" tobaccos and with "processed smoking materials" (artificial tobacco). Paper of high porosity is favorable and current filter experiments appear of interest in reducing gas phase components such as carbon monoxide and nitrogen oxides.

The evaluation of cigarettes would be more physiologic if the smoke could be given to animals by inhalation. Concentrated development in this area has led to successful design of effective inhalation tests in small animals, now ready to be used for the assessment of experimental cigarettes.

The limitations of condensate evaluation by dermal assay in mice as a model of exposure of human lungs to whole smoke are recognized. A major effort is underway to develop inhalation assays and extend them to more relevant animal models. Work has been conducted for some time with small rodents and beagles. New projects have recently been advertised for new work on beagles and baboons, and for tests with normal volunteers. Accomplishments include successful chronic exposure of hamsters and rats, and of beagles via tracheostoma. Machines have been devised for standardized smoking of cigarettes and delivery of controlled doses to the animals; routines have been worked out to maximize dosage despite interfering acute toxic effects.

Other bioassays are continually sought to evaluate experimental cigarettes and study the etiology of adverse health effects. Models of all aspects are needed, and rapid, accurate response is desired. Techniques now or recently under development include: lung pellet implantation; alveolar macrophage inhibition; mucociliary activity inhibition; cell culture growth processes; and intratracheal and intragastric administration to rats. A technique of possible potential is dorsal grafting of tracheal segments in rats and hamsters which provides accessible respiratory tissue for tests otherwise impractical. New observational techniques for animals under chronic exposure emphasize the development and interrelation of bronchitis and emphysema, and cardiovascular effects related to atherosclerosis.

A wide literature survey and inquiry is underway to identify the most promising methods for estimating dose in smokers.

Gratifying progress has been made in the identification of specific carcinogens in smoking products; most recently nitrosonornicotine. Increased emphasis in the study of promoters has been imitated.

Protection of the smoker includes current work on identification of high-risk individuals by enzyme induction assay and by metabolic idiosyncrasies, and investigation of nicotine agonists and antagonists to reinforce smoking cessation counseling.

Current and Planned Research

Current research is designed to confirm the initial results, extend their significance into the design of new cigarette models and insure that improvements related to the carcinogenicity of smoke condensate do not worsen other characteristics of the cigarette, particularly those affecting the cardiovascular and respiratory systems. In this context and in team with the National Heart and Lung Institute consultants, methods have been evaluated to determine the cardiovascular and respiratory effects of experimental cigarette smoke in man and experimental animals.

Also, in team with the United States Department of Agriculture, research efforts have been defined to develop agronomic varieties of tobacco that may result in less hazardous smoking products.

Research projects now proceeding or to be implemented during fiscal year 1974 include the following:

1. Skin painting, ciliatoxic and cytotoxicity bioassay of less hazardous cigarette models. These include 60 new experimental cigarettes with various additives, papers of different porosity, reconstituted tobacco sheets of different densities and nature, processed cellulose artificial smoking materials, high and low nicotine tobaccos, hexane extracted tobaccos, etc., as suggested by the correlation of chemical and biological data from the initial experiments.

2. Chemical analysis of experimental cigarettes.

3. Development of novel bioassay techniques such as pellet implantation technique in rodent lungs.

4. Development of inhalation methods in small rodents, dogs and baboons.

5. Determination of chronic nicotine effects by inhalation experiments in dogs.

6. Development of experimental methods to measure smoke dose intake in man.

7. Inhalation bioassay of experimental cigarette smoke dose intake in man.

8. Measurement of acute bronchitis effects in man with various experimental cigarettes.

9. Respiratory impairment in beagles exposed to cigarette smoke.

10. Clinical trials of pharmacological approaches in smoking withdrawal.

11. Metabolic studies of tobacco smoke constituents.

12. Standardization of aryl hydrocarbon hydroxylase assay as a screening method.

13. Chemical modeling of cigarette combustion dynamics.

14. Epidemiologic surveys to determine multifactorial causes of disease associated with smoking; these may include occupations, social habits, diseases, genetic and metabolic characteristics of the smoker, such as demographic and social variations, occupational and environmental characteristics, smoking habits and inhalation characteristics, and smoking products used (cigarette vs. cigars and pipes).

Program Management and Organization

The Smoking and Health Program is directed by Dr. Gio B. Gori, Acting Deputy Director, Division of Cancer Cause and Prevention, and a Program Manager, Dr. Thomas B. Owen. There is no inhouse research staff; Enviro Control, Inc. (ECI) provides research management services and is developing into a prime contractor role with executive responsibility for many facets of the program including subcontracting.

CONTRACT NARRATIVES

SMOKING AND HEALTH PROGRAM

AEC-OAK RIDGE NATIONAL LABORATORY (Y01-CP-40207)

Title: Collection, Separation, and Elucidation of the Components of Cigarette Smoke. Part I - Chemical Characterization of Cigarette Smoke. Part II - Cigarette Smoke Bioassay Development.

Contractor's Project Directors: Part I - Dr. Michael R. Guerin
Dr. Wilbur D. Shults
Part II - Dr. Paul Nettesheim
Dr. Michael R. Guerin

Project Officer (NCI): Dr. Gio B. Gori

Objectives: Part I - To generate data defining the chemical characteristics of experimental cigarettes undergoing biological testing and to support related studies by other contractors as deemed necessary by the NCI.

Part II - To develop new methods for assessing the relative biological impact of cigarette smokes.

Major Findings: Part I - Chemical data defining the composition of the first series of NCI experimental cigarettes (23 variants) has been correlated with independently generated skin painting results. The finding of a high correlation with isoprene has rekindled interest in terpenoid contribution to biological activity. Total free fatty acids were also found well enough correlated with biological results to warrant their incorporation in routine analytical protocols. Trace metals, including nickel, were found poorly correlated with biological results.

Data has been generated for the deliveries of acetaldehyde, acrolein, isoprene, formaldehyde, hydrogen cyanide, oxides of nitrogen, carbon monoxide, carbon dioxide, total particulate matter, nicotine, water, and tar by each of the second series of 25 experimental variants. Condensate components (nicotine, phenol, o-cresol, m-cresol plus p-cresol, weak acids, very weak acids, palmitic acid, oleic-linoleic-linolenic acids, stearic acid, indole, skatole, benz(a)anthracene, benzo(a)pyrene, and pH) have been measured in three sets of samples. The fourth and final set will be completed shortly.

Analytical chemical research and development efforts have produced computer methods allowing graphical representations of smoke composition and chemical-biological correlation plots. The methods offer considerable promise for simplifying data studies. A procedure based on nitrogen selective gas chromatography for the quantitative determination of NO has been developed which may supplant the classical determination of NOx. A procedure for the quantitative determination of indole and skatole in condensates has been developed and is in routine use. Continued studies of component profiling have demonstrated the possibility of examining as many as 200 gas phase components for biological activity.

Collaborative studies with Arthur D. Little, Inc. have demonstrated the reliability of a new device for intratracheally exposing dogs to whole cigarette smoke. Preliminary study of dosimetry data suggest that doses received using the new device are comparable to those received by direct dog puffing.

Part II - The intra-laryngeal cannula has been judged ineffective for chronic smoke inhalation exposure because of intolerance to long term cannulation. The cannula is found promising, however, for short term exposures.

Increasing smoke stand time from thirty to forty seconds for each puff is found to produce disproportionately increased dose to hamsters. Spacing the cigarette exposures to allow mild detoxification allows exposing hamsters to as many as 10 cigarettes per day using the 40 second smoke stand time. The result is that the dose is more than doubled compared to the best protocols used by other workers. Further studies have demonstrated that it is possible to expose rats to as many as seven cigarettes per day with proper smoke conditioning.

Preliminary study of existing data suggests that the greater tolerance to smoke inhalation exhibited by one strain of hamsters is artificial; the respiratory pattern is sufficiently different from that of the other strain tested to produce a lower total dose received per cigarette.

It has been found possible to produce a viable tracheal graft. The graft trachea serves as a source of respiratory epithelium which can be contacted with carcinogen-containing pellets. Studies of smoke condensate using this technique are underway. It may also be possible to route whole smoke into or through the graft; substantially increased doses may be possible.

Initial studies of the chemistry of the smoke in the inhalation chamber suggest that no significant change in composition occurs during standing,

but highly reactive components and particulate matter composition must be studied before final conclusions are possible. The presence of animals does affect the composition of the aerosol. Carbon monoxide and nitric oxide are depleted significantly and carbon dioxide increases. Quantitative reductions of nicotine and of gas phase organics generally parallel expectations based on tracer dosimetry studies.

Hardware development has produced a substantially more reliable and versatile smoke generation-animal exposure machine. The system has now been modified to allow exposing rats as well as hamsters. Adequate animal containment devices are now available for both rats and hamsters.

Significance to Biomedical Research and the Program of the Institute: The National Cancer Institute has assumed much of the responsibility for defining the carcinogenic potential of cigarette smoking and steps which might be taken to reduce that potential. Chemical studies provide the methods and data by which biological activity may be related to characteristics of the smoke. Bioassay studies provide methods which will allow a more reliable definition of the relative biological activities of products under test.

Proposed Course: Part I - To continue providing the chemical data and methods necessary to characterize the products under test.

Part II - To finalize the most promising inhalation method and apply it to study the relative carcinogenicity of experimental products while evaluating the tracheal graft as an alternate method.

Date Contract Initiated: Part I - November, 1969
Part II - July, 1971

Current Annual Level: Part I - $400,000
Part II - $756,000

AMERICAN HEALTH FOUNDATION (NO1 CP-33305)

Title: Part I - Epidemiologic Evaluation of Carcinogenic Agents in Cigarette Smoke

Contractor's Project Directors: Ernest L. Wynder, M. D.
Kiyohiko Mabuchi, M. D.

Project Officer (NCI): Dr. Gio B. Gori

Objectives: To determine the means of preventing diseases related to tobacco usage by identifying less hazardous smoking products and habits, and susceptible subjects. The effect of changes in tobacco products on risks of cancers of the respiratory tract, the upper alimentary tract, the urinary tract, the pancreas, and the biliary tract will be measured.

Major Findings: As of September, 1973, 13,830 hospitalized individuals were interviewed at selected hospitals in New York City, Los Angeles, Houston, Birmingham, Miami and New Orleans.

The present study substantiates a previous finding that individuals who have smoked filter cigarettes at least ten years after switching from nonfilter cigarettes (long-term filter smokers) have a lower risk of lung cancer than those who continued to smoke nonfilter cigarettes. The study continues to determine whether those who have smoked only filter cigarettes have a further reduced risk of lung cancer. Future studies will determine cancer risk by cigarette tar level.

Large sex differences appear among groups smoking at different intensities at ages 50 $\pm$ years, but are greatly diminished at younger ages due to the recently increased smoking exposure of young females. This suggests that lung cancer mortality will continue to increase in U.S. females. The rate will not achieve the current high male level because female cigaratte smoking has only become widespread in recent years and female smokers use more filter/low tar cigarettes than male smokers.

Higher levels of education and being Jewish were both found to be associated with greater percentages of nonsmokers, ex-smokers, and filter/low tar cigarette smokers, suggesting that tobacco-related diseases may become increasingly uncommon in highly educated groups and continue to be uncommon in the Jewish population.

The study continues to substantiate preliminary findings on reduced risks of cancers of the vocal cord, oral cavity, esophagus and bladder, among male long-term filter cigarette smokers.

The study also continues to assess the possible hazards of the recently-introduced little cigars which may be inhaled as easily as cigarettes.

Significance to Biomedical Research and the Program of the Institute: The NCI has the major responsibility of defining the hazards of smoking. Resources capable of performing epidemiologic surveys in this field with updated chemical and biological information are limited. Biochemical information is readily available from laboratory studies currently in progress at the American Health Foundation. Human information is of vital

importance and provides the final judgment about which cigarettes are less hazardous.

Proposed Course: Additional opportunities for expansion and continuation of scientific efforts by the contractor have been identified, should the funding situation improve.

Date Contract Initiated: February 24, 1970

AMERICAN HEALTH FOUNDATION (NO1 CP-33305)

Title: Part II - Evaluation of Carcinogenic Agents in Cigarette Smoke Chemical and Biological Assays

Contractor's Project Director: Dr. Dietrich Hoffmann

Project Officer (NCI): Dr. Gio B. Gori

Objectives: Isolation, identification and reduction of carcinogens, tumor initiators and tumor promoters in tobacco and tobacco smoke. Development of an inhalation technique for the assay of tumor promoting activity of cigarette smoke.

Major Findings: We have shown for the first time that tobacco and tobacco smoke contain nonvolatile N-nitrosamines and hydrazine. During curing and burning the tobacco alkaloids serve as precursors for nonvolatile N-nitrosamines. The most important one, nitrosonornicotine, is primarily formed by dealkylation and nitrosation of nicotine and only to a minor extent by nitrosation of nornicotine as shown by studies with ^{14}C-labelled nicotine. The carcinogenic nitrosonornicotine is found in all tobacco and tobacco smoke products including chewing tobacco.

Tobacco smoke contains a large spectrum of alkylated polynuclear aromatic hydrocarbons (PAH). Alkylfluoranthenes and alkylchrysenes contribute significantly to the tumor initiating activity of tobacco smoke. The six isomeric methylchrysenes were synthesized and bioassayed. Chrysene itself and all six methylchrysenes are active as tumor initiators; 1- and 4-methylchrysene are inactive as carcinogens; chrysene and 2-, 3-, and 6-methylchrysene are weak carcinogens; and 5-methylchrysene is a very strong carcinogen.

The weakly acidic portion of tobacco smoke condensate is active as a tumor promoter. Fractionation experiments led to a subportion which is active as tumor promoter and which contains N-alkylated amino-phenols,

catechol, alkylated catechols, and long-chain fatty acids.

Tobacco terpenes contribute significantly to the overall tumorigenicity of tobacco smoke by serving as precursors for carcinogenic PAH, for PAH which accelerate the carcinogenicity of active PAH, and for neutral tumor promoters. Detailed studies are currently under way.

Analytical methods were developed for maleic hydrazide, hydrazine, nitrosonornicotine, chrysenes, carbon monoxide, carbon dioxide, pH of total smoke and for the PAH profile of tobacco smoke.

Chemical-analytical studies combined with the tests on a smoke panel suggest that "tar", pH, carbon monoxide and ammonia are the predominant factors which determine the degree of "inhalability" of the smoke of cigarettes and little cigars.

Inhalation experiments have shown that tobacco smoke can promote the induction of tumors in the larynx of Syrian golden hamsters which were initiated with a low dose of a carcinogenic hydrocarbon. At present the system is used for comparing the promoting activity of the smoke of various cigarettes.

Significance to Biomedical Research and the Program of the Institute: It is estimated that in 1973 more than 60,000 Americans will have died from lung cancer; the majority of these will have been heavy cigarette smokers. Whereas the best way to reduce the risk of lung cancer is to give up smoking, only a few smokers are able and are willing to do so. This places major importance on the reduction of the carcinogenic potential of tobacco smoke. A less harmful cigarette can be realized with the identification and reduction of tumorigenic and carcinogenic agents in tobacco and tobacco smoke, the major goal of this research contract.

Proposed Course: To continue as outlined above.

Date Contract Initiated: February 24, 1970

Current Annual Level: $748,900

AMERICAN HEALTH FOUNDATION (YO1 CP-20205)

Title: Study of Smoking Intervention Techniques

Contractor's Project Director: Selwin Waingrow

Project Officer: Daniel Horn, M. D.

Objectives: Investigation of the efficacy of various smoking intervention techniques and the relationships between success and failure, techniques used, and personality and smoking characteristics of subjects. Work is being conducted in two major areas: minimal interventions in routine procedures and more-maximal clinic-type interventions, both group and individual. Study is intended to clarify variables indicative of success with minimal intervention as opposed to more intensive approaches.

To date, the two areas have been developed separately: minimal intervention subjects are smokers showing no particular interest in stopping smoking, while clinic subjects are relatively highly motivated. The results will now be synthesized and intermediate procedures will be developed and tested.

Major Findings: The crucial work of relating and contrasting levels of intervention is nearing completion. Some of the failures at one treatment level have been assigned to treatment at a more intense level. Independently, those same intense treatments have been given to new subjects for whom this will be the first intervention. In this way, multiple comparisons are now possible between failures at one level leading to success at a later level as opposed to those also failing at a later level, as compared against those who received the later level intervention as a first intervention.

Significance to Biomedical Research and the Program of the Institute: These variables and success-failure data in a variety of interventions at a variety of intensity levels will provide much needed information along many dimensions simultaneously. Questions such as: What treatments work best for women? What characteristics of women contribute toward their relative difficulty with certain attempts to stop? and so on, may all be fruitfully approached in such a format.

Proposed Course: Questionnaire, interview, treatment, and follow-up data will be analyzed. The variables to be studied will include: demographic (age, sex, socio-economic status, religion, education, etc.), attitudes toward smoking and toward smoking cessation, attitudes toward authority, peers, competition, orality, nurturance, etc., smoking behavior (intensity, activities associated with smoking, etc.), comprehensive medical history variables, attitudes toward preventive health care and risk-taking behaviors, and associated material related to smoking and health.

Date Contract Initiated: June 24, 1972

Current Annual Level: $160,000

BATTELLE PACIFIC NORTHWEST LABORATORIES (NO1 CP-33374)

Title: Research on Standardizing Lung Pellet Implantation Technique in the Rat

Contractor's Project Director: Dr. Gerald E. Dagle

Project Officers (NCI): Dr. Thomas B. Owen
Dr. Mearl F. Stanton

Objectives: To evaluate a beeswax-tricaprylin-cigarette smoke condensate lung pellet implantation technique to standardize the technique for the bioassay of select cigarette smoke condensates and to determine if such standardization may allow a shorter period for the bioassay of these condensates than other presently used bioassay techniques.

Major Findings: A four-week preliminary study in Osborne-Mendel rats showed squamous metaplasia associated with the implantation site was more severe with increasing size of pellets and higher concentration of condensate. Sprague-Dawley rats were implanted with lung pellets and will be sacrificed after 120 weeks. Osborne-Mendel rats currently are being implanted with various size pellets having different concentrations of two different cigarette smoke condensates, and will be sacrificed 10, 30, 60, 90, and 120 weeks after implantation.

Significance to Biomedical Research and the Program of the Institute: The induction of epidermoid carcinomas in the lungs of rats by implantation of relatively small amounts of cigarette smoke condensate in beeswax-tricaprylin-cigarette smoke condensate pellets in the lung (Stanton and co-workers) suggests that the lung pellet implantation technique could be used for routine cigarette smoke condensate carcinogenicity bioassay in the development of a less hazardous cigarette if the technique could be standardized. The potential advantages of this bioassay system would be that only one injection is required and the lung, the major concern in the human smoker, is the target organ.

Proposed Course: Histopathologic evaluation will proceed as the rats are periodically sacrificed. A recommendation will be made on the optimal size pellet and concentration. Pre-neoplastic changes will be evaluated in an effort to shorten the bioassay time period.

Date Contract Initiated: June 30, 1973

Current Annual Level: $97,262

ENVIRO CONTROL, INC. (NO1 CP-33382)

Title: Research Management Services to the Smoking and Health Program

Contractor's Project Director: Mr. A. E. Hayward

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: To provide technical and managerial support. Specific tasks include: analysis and evaluation of data from bioassays; preparation of working papers and reports for scientific conferences; preparation of technical RFPs; site visits and progress report reviews; logistics support for major interagency cooperative investigations; budget analysis; procedures for research advisory and evaluation boards; writing review reports on biomedical investigations.

Major Findings: A new method of profile analysis has refined and effectively displayed correlations between tumorigenic potency components of tobacco, smoke and condensate. Analysis of dose response relationships has contributed to experimental design and evaluation. Experimental protocols have been compiled for cardiovascular and pulmonary investigations.

Significance to Biomedical Research and the Program of the Institute: The significance of this segment is inseparable from that of the whole Smoking and Health Program, with which this contract is involved throughout: specifically, in such activities as the correlation of carcinogenicity with components of tobaccos and smoke, identification of chemical bases of carcinogenicity, elucidation of respiratory retention and dosimetry, and studies of the progression of acute bronchitis, chronic bronchitis, and emphysema. The contractor's most significant focal role is in the recommendation of methods for bioassay, experimental design, and application of distribution-free statistical analysis.

Proposed Course: The contractor will continue present services and assume wider responsibilities during 1974: staff assistance to the Director, Smoking and Health Program, in defining and evaluating development projects; Prime Contractor role for all nonfederal subcontracts; more limited responsibilities for Interagency Agreements; logistics for large cooperative experiments; data analyses. The present contract runs through June 30, 1976.

Date Contract Initiated: July 1, 1973

Current Annual Level: $740,497

HAZLETON LABORATORIES (NO1-CP-23275)

Title: Chronic Carcinogenesis Bioassays by Intratracheal Instillation of Cigarette Smoke Condensates in Syrian Hamsters

Contractor's Project Director: Dr. Marcelina B. Powers

Project Officers (NCI): Dr. Michael B. Sporn
Dr. John A. Cooper

Objectives: To evaluate the carcinogenic effects of various cigarette smoke condensates in hamsters resulting from repeated intratracheal instillation.

Three cigarette smoke condensates were supplied by Meloy Laboratories under contract to NCI (NIH-NCI-69-2084).

Major Findings: The condensates were administered by intratracheal instillation in Syrian hamsters once weekly for 50 weeks. Survival was generally low for all groups including controls during the 50-week treatment and the 30-week observation periods. No tumors were observed in the trachea, primary bronchi or lungs of animals that died or of survivors killed terminally. Histopathology of tissue sections from selected animals did not reveal any histologic alterations indicative of preneoplastic or neoplastic lesions.

Significance to Biomedical Research and the Program of the Institute: In previous investigations bronchogenic tumors have been induced intratracheally in hamsters with benzo(a)pyrene and other chemical carcinogens. The use of the technique for bioassay of tobacco condensates was severely limited by the dose size, physical consistency and toxicity of the test materials. The results serve as a background against which a more definitive experimental protocol could be designed in future studies.

Proposed Course: This program has been completed and the contract will terminate during this support period. A final report will be submitted.

Date Contract Initiated: May 20, 1971

Current Annual Level: $59,269

HAZLETON LABORATORIES (NOI-CP-92149)

Title: Skin Carcinogenesis Bioassay of Cigarette Smoke Condensates in Mice

Contractor's Project Directors: Mr. James L. Gargus
Dr. Marcelina B. Powers

Project Officer (NCI): Dr. Gio B. Gori

Objectives: To provide a standardized bioassay for the determination of the carcinogenicity of cigarette smoke condensates by repeated dermal application in mice.

Whole smoke condensates are supplied by Meloy Laboratories under NCI Contract (NIH-NCI-69-2084). The experimental variables include tobacco of different origins; methods of cultivation, including fertilization, suckering agents, and soils; curing and aging methods, blends of tobacco plant parts such as leaves, stalks, and stems; reconstituted tobacco, cutting of tobacco, cigarette paper, and filters.

Female ICR mice are treated six times weekly with dermal applications of the whole smoke condensates dissolved in acetone. Skin tumors are recorded and measured. All animals are necropsied and tissues are fixed and preserved for histopathological examinations. Data on each animal are entered in a computer memory once monthly. Computations and printed reports from NCI provide monitoring data for continuous evaluation of the experimental results.

Major Findings: The mouse skin bioassays of the first series of 26 experimental condensates have been completed. Histological findings have been reported and are being correlated with the chemical analysis of the condensates.

A second series of dermal bioassays of 25 different smoke condensates were initiated in February, March, and April, 1972. The experimental design employed in this series was the same as that previously employed. All animals were sacrificed after the 18-month experimental period. Histopathological examination of all skin tumors is in progress and will be completed in February, 1974.

A third series of mouse dermal bioassays of approximately 30 smoke condensate variables will be initiated in March - April, 1974.

Significance to Biomedical Research and the Program of the Institute: The comparative bioassay results along with other monitoring bioassays and chemical determinations will permit evaluation of the biological effects of the variables in the different smoke condensates.

These comparative evaluations will provide data for testing and developing less hazardous cigarette formulations.

Proposed Course: Complete the tumor examinations and analysis of data derived from the second series of dermal bioassays and initiate the third series in March - April, 1974.

Date Contract Initiated: June 24, 1969

Current Annual Level: $298,573

HAZLETON LABORATORIES (NO1-CP-92145)

Title: Carcinogenicity Bioassays by Intragastric Intubation of Cigarette Smoke Condensates in Experimental Animals

Contractor's Project Director: Mr. James L. Gargus

Project Officer (NCI): Dr. John Cooper

Objectives: The evaluation of the carcinogenic potential of cigarette smoke condensates following repeated oral administration in rodents.

Experimental variables in the smoke condensates includes nicotine content and total dose. If a satisfactory bioassay can be standardized using the oral route of administration, it will be utilized in the comparison of tobacco condensates of experimentally modified cigarettes. These bioassays are a part of the research program directed toward the development of a less hazardous cigarette.

The cigarette smoke condensates have been furnished by Meloy Laboratories (NIH-NCI-69-2084) under contract to NCI.

Major Findings: Two tobacco condensates were administered at 15 mg/kg and 25 mg/kg to mice for 18 months and to rats for 15 months. No gross observations of compound-induced carcinogenic effects were observed. The nicotine content of the condensates induced toxicity and excessive mortality at levels of administration higher than 25 mg/kg.

All animals were sacrificed in March, 1973. Selected tissues (lungs, stomach, pancreas, and urinary bladder) were prepared, for histological examination by National Cancer Institute personnel.

Significance to Biomedical Research and the Program of the Institute: This project is an integral part of the program for the formulation of a less hazardous cigarette. This program requires that new bioassay techniques be devised which will be both sensitive and specific. The selection of this route of administration for emphasis is justified by the large quantity of tobacco tars and condensate which are ingested into the stomach in the course of smoking and subsequent ciliary clearance of the respiratory tract. The toxicity of the nicotine in the condensate is a significant limiting factor in a bioassay procedure utilizing the oral route of administration.

Proposed Course: This study has been terminated and no additional experimental work is anticipated.

Date Contract Initiated: June 24, 1969

Current Annual Level: $49,200

ARTHUR D. LITTLE, INC. (NO1 CP-33349)

Title: Measurement of Tobacco Smoke Components in Human Subjects

Contractor's Project Director: Dr. Sam P. Battista

Project Officer (NCI): Dr. Gio B. Gori

Objectives: To review the literature and interview expert consultants to determine which smoke component(s) in physiological fluids correlate quantitatively with smoke intake in human subjects.

Major Findings: To date we have compiled and are reviewing more than 500 publications to establish not only the compounds reported in cigarette smoke, but also the amount and methodology for their analysis. Also sought are smoke components measured in body fluids in both smokers and nonsmokers. Special analytical techniques -- some still in the experimental stage -- are being evaluated for possible use. The analytical methods described in the smoking literature are unsatisfactory due to either lack of sensitivity, the pharmacokinetic uncertainties of the agent, or the time and effort required for sample preparation.

Significance to Biomedical Research and the Program of the Institute: Analysis of past epidemiologic studies to establish the relationship between the level of cigarette smoking and various diseases has been confounded by the great variability in the smoking habits of the individual smoker. The development of a suitable method for estimating the actual dose of smoke inhaled, instead of subjective estimates of the number of cigarettes smoked, will provide more precise and reliable data to assess the health hazards of cigarette smoking.

Proposed Course: The present contract will end April 30, 1974. At that time a list of the most promising methods will be provided along with a selection of the most likely candidate and the rationale for its selection.

Date Contract Initiated: June 29, 1973

Current Annual Level: $66,732

ARTHUR D. LITTLE, INC. (NO1 CP-33284)

Title: Bioassay of the Cytotoxicity of Cigarette Smoke and of Its Effects on Ciliary Function

Contractor's Project Directors: Dr. Philip S. Thayer
Dr. Sam P. Battista

Project Officer (NCI): Dr. Gio B. Gori

Objectives: (1) To evaluate experimental cigarettes in two test systems: (a) Effects of smoke on growth and phagocytic activity of *in vitro* cultivated cells; (b) Effects of smoke on ciliary kinetics and dynamics of mucus transport in the respiratory epithelium of experimental animals *in vitro* and *in vivo*. (2) To develop and evaluate a model system to determine whether cigarette smoke has effects on carcinogenesis in the chicken trachea as influenced by experimental removal of the tracheal epithelium.

Major Findings: Bioassays - (1) Results for 23 cigarette variables showed significant correlations between one or more biological activities and content of acetaldehyde, formaldehyde, hydrogen cyanide and/or acrolein. (2) Twelve filter cigarettes have been assayed in the two cell systems and against cilia *in vitro*. Results for inhibition of cell growth and ciliatoxicity correlate well with each other and with content of HCN, and to a lesser extent of acrolein. (3) A further 24 cigarette variables have been assayed against the cell systems and cilia *in vitro*, and the data are being analyzed and correlations examined.

Neoplasia Model - These studies were set up to examine the long-term effects of exposing chickens to cigarette smoke alone, the carcinogen diethylnitrosamine (DEN) alone, and a combination of DEN and cigarette smoke. During the initial twenty weeks of testing half of the animals received mechanical denudation of the trachea. Cytological examination of the exfoliated cells harvested during successive denudations showed progressive increases (followed in some cases by decreases) in nuclear atypia, active and reactive chromatin and metaplastic changes. They were most pronounced in the smoke and smoke + DEN groups, although DEN alone produced significant increases. Smoke treatment is continuing and the significance of these changes must await assessment of the longer term results. Histological examination of lungs, tracheas and air sacs of those animals which died on test and those sacrificed at one year have given preliminary indications of pre-neoplastic changes and scattered incidence of malignancies.

Significance to Biomedical Research and the Program of the Institute: The overall program for the formulation of a less hazardous cigarette needs to identify physiological responses of the respiratory tract surface tissues to cigarette smoke of different characteristics.

This information will be correlated with carcinogenesis and chemical data in testing and directing modification of cigarettes towards less hazardous formulations.

Proposed Course: The four bioassay procedures are available for evaluation of subsequent sets of experimental cigarettes. The development and evaluation of the neoplastic model system is proceeding, and further experimental animals may be sacrificed during the year.

Date Contract Initiated: June 19, 1969

Current Annual Level: $89,237

MELOY LABORATORIES, INC. (NOI-CP-92084)

Title: Preparation and Analysis of Cigarette Smoke Condensate Samples

Contractor's Project Director: Dr. A. R. Patel

Project Officer (NCI): Dr. Gio B. Gori

Objectives: To machine-smoke experimental cigarettes in large numbers, under closely controlled conditions, to prepare smoke condensates for skin painting assays, other bioassays, and chemical analysis; to carry out a quality control program of chemical analysis on all batches of cigarette smoke condensate both to insure uniformity and to characterize the product to some extent for the subsequent users.

Major Findings: This is a complex service contract, the scope of which is production of cigarette smoke condensate on schedule and involves quality control on each batch of smoke condensate.

Significance to Biomedical Research and the Program of the Institute: This contract is designed to produce the cigarette smoke condensate required for the execution of three other projects which are funded under the Lung Cancer Task Force: (1) Skin Carcinogenesis Bioassay of Cigarette Smoke Condensate, (2) Skin Carcinogenesis Bioassay of Marijuana Cigarette Smoke Condensate, (3) Chemical Analysis of Cigarette Smoke Condensate Samples.

Proposed Course: Activities of this contract started in February 1972, as experimental cigarettes became available. Analytical work on the unsmoked tobacco from these experimental cigarettes is being carried out. Production activities on the third series of NCI cigarettes will proceed on schedule.

Date Contract Initiated: June 30, 1969

Current Annual Level: $453,272

VETERANS ADMINISTRATION HOSPITAL, EAST ORANGE, NEW JERSEY
(YO1-CP-40205)

Title: Effects of High and Low Nicotine Cigarettes on Male Beagle Dogs

Contractor's Project Director: Oscar Auerbach, M. D.

Project Officer (NCI): Dr. Gio B. Gori

Objectives: (1) Compare the effectiveness of two different methods of delivering cigarette smoke to beagle dogs; (2) Test and evaluate a smoking machine designed by Dr. Sam Battista of Arthur D. Little, Inc.; (3) Conduct a pulmonary deposition study using 14-C dotriacontane labeled cigarettes; (4) Based on results of the above studies, the major study was initiated, which is to determine the significance of the nicotine content of

cigarettes in the development of pulmonary and cardiovascular disease during chronic smoking, using male beagle dogs.

Major Findings: (1) The direct method of smoking, used earlier at this laboratory, was found to have considerable variability from one dog to another, and more risk to the dog. The ADL-designed machine was found to be safe and the delivered dose was uniform.

Histopathologic study of lung tissues from dogs exposed in both ways for up to eleven months demonstrated that there were smoking-associated changes in the tracheobronchial epithelium as well as some parenchymal changes. Most significantly there was focal basal cell hyperplasia and early developing areas of squamous metaplasia in the epithelial lining of the major bronchi and trachea. This was most noticeable in those dogs which smoked for nearly a year (343 days). Some tracheobronchitis was present. There was focal areas of thickening of interalveolar septa due to collagen deposition. There were focal areas of early emphysematous changes, consisting of rupture of interalveolar septal walls. There was mild alveolar macrophage proliferation and foci of bronchioloalveolar epithelial hyperplasia. (2) The ADL machines have been in daily operation for as long as seven months and appear to be satisfactory for this project. It has been our experience that one good technician can simultaneously operate five machines without much difficulty. (3) The dosimetry study, done in collaboration with Oak Ridge National Laboratory used thirty dogs. The dogs were smoked, at a maximum of eleven cigarettes each day, for time periods of up to six months, using a commercial brand of cigarettes. At the termination of the smoking phase of the study, the dogs each smoked five cigarettes followed by one 14-C dotriacontane spiked cigarette, using (a) the direct method, (b) the ADL machine at 6, 3 and 2 puffs/min. The dogs were immediately killed, the trachea ligated, and the following tissues removed for analysis: larynx, trachea, lungs, esophagus, stomach, cardiac blood. Tissues were immediately frozen and were transported to Oak Ridge National Laboratory for digestion and analysis. This study was completed in December, and the data has not been completely derived and evaluated as yet. Early evaluation of the data indicated that the ADL machine was at least as effective in delivering cigarette smoke to the pulmonary tissues as the previously used direct method. (4) Based on indications from (2) and (3), the chronic study was initiated in January, using the ADL smoking machine and one hundred and twenty tracheostomized male beagle dogs.

Significance to Biomedical Research and the Program of the Institute: These experiments have helped to develop a standardized smoking procedure, in which a reliable model system of dog and machine can be used and the uniformity of delivered cigarette smoke to each dog can be well

controlled. The collaborative dosimetry study with Oak Ridge National Laboratory may provide some of the best currently available data concerning cigarette smoke product deposition in the respiratory system of an experimental animal. The high and low nicotine cigarette smoking study will provide information as to the role played by nicotine in disease states associated with cigarette smoking. These include emphysema, cardiovascular disease, bronchitis, and pulmonary neoplasia. If nicotine is involved in the pathogenesis of these disease, some indication should be manifested after 1,000 days of smoking by these beagle dogs.

Proposed Course: To continue the program as described in the research proposal.

Date Contract Initiated: April 1, 1972

Current Annual Level: $229,290

REQUEST FOR PROPOSALS

SMOKING AND HEALTH PROGRAM

RFPs Now Being Processed

RFPs for nine new projects are being written, evaluated and approved.

Five of them cover aspects of the development and use of various animals in the evaluation of experimental cigarettes, part of the less-hazardous cigarette program. The chronic effects of inhaling cigarette smoke are to be studied in a large-scale experiment with rats and two large experiments with dogs; one of the dog experiments emphasizes the non-neoplastic hazards of tobacco smoke. Work is to be done on the baboon, a closer model of man in which atherosclerosis can be induced for better simulation of the high-risk smoker. Acute bronchitis is to be studied in volunteer novice smokers by a technique known to show measurable response to a single cigarette.

The remaining RFPs relate to aspects of protecting the smoker: metabolic study of health hazard mechanisms and defense against them; identification of high-risk individuals; drugs to assist in cigarette withdrawal; and assessment of the acceptability to the smoker of less-hazardous cigarettes.

A brief description of each new project follows.

Inhalation Bioassay of Cigarette Smoke in Rats. This project will accelerate the trend towards inhalation assay which is theoretically a more realistic model of lung cancer hazards than mouse skin painting and also gives opportunity to study other pulmonary and systemic effects. It will apply the inhalation assay to five key types of experimental cigarettes, thus advancing the development of less-hazardous cigarettes. Techniques for inhalation exposure of small rodents are quite well developed (especially at Oak Ridge National Laboratories) but they are some way yet from being a standardized operations-manual methodology: narrowing this gap is another object of the contract.

The contractor will set up and demonstrate his capability to embark on this large and demanding experiment. He will then expose daily five groups of 120 rats each to smoke from the experimental cigarettes, with suitable controls. Rats will be sacrificed at three-month intervals, from the sixth month to termination at two years. Non-invasive tests of respiratory function will be made on live rats, and post mortem observations will

emphasize histopathologic examination for neoplastic processes and bronchitis/emphysema.

Inhalation Bioassay of Cigarette Smoke in Dogs: High and Low Nicotine Content. This is similar to the rat inhalation project in being directed both to the development of an important animal model and to further study of a critically important problem -- evaluating the health effects of nicotine. It also resembles the rat inhalation project in studying not only carcinogenesis but respiratory and other effects, for which the beagle is an excellent subject, and it greatly extends the existing study in beagles to other respiratory effects, provision of dose response data, and collateral physiological and biochemical parameters.

Exposure will be via tracheostoma, with a cigarette-smoking machine and daily routine which are already in use. Groups of 60 dogs each will receive smoke from high-nicotine and low-nicotine cigarettes at two dose levels, requiring -- with controls -- 300 beagles. Since pulmonary effects such as emphysema are known to be well developed at 450 days, while significant neoplasia is not to be expected before 1000 days, the experiment will provide for sacrifice at these (and other) times, as well as routine pulmonary and metabolic tests on the live animals. Nicotine is important because its concentration in smoke condensate correlates with tumorigenicity in mouse skin painting; it has also been implicated in various cardiovascular effects. Post mortem examination will look for pulmonary neoplasias, other pulmonary effects, and cardiovascular changes especially in the myocardial arterioles.

Respiratory Impairment in Beagles Exposed to Cigarette Smoke. This is an exploratory development, unlike the previous one, and its objectives are quite different: to develop a quick-response screening test for experimental cigarettes, and as the first step in developing an early indicator of smokers with high risk of emphysema. The approach will exploit the technical and other advantages of the beagle as an experimental animal and in particular will attempt to apply closing volume measurements which are known to be a sensitive indicator of small airway impairment in humans. The closing volume test has not been adapted to the beagle and this is the first task. Whether or not it is accomplished, other candidates for quick screening are sought, together with development of techniques for applying them to the beagle. The long term exposure will include routine pulmonary function tests on the live animals and serial sacrifice with detailed pulmonary and cardiovascular histopathology. The experiment offers opportunities to look for concurrent effects of chronic smoke exposure, including study of hemostasis, and for precursors and development of neoplastic processes which might occur late in the experiment. A specific task for the contractor is to study closely the succession of acute bronchitis,

chronic bronchitis and emphysema which might make one a useful predictor of the next.

The long term exposure will not be undertaken unless the preliminary attempt to apply closing volume and other measurements is sufficiently encouraging.

Feasibility of Inhalation Testing of Cigarette Smoke in Baboons. This project will evaluate the baboon as an experimental animal for screening cigarettes; the work will also be valuable to investigators of the etiology of smoking-related disease who wish to use baboons.

The baboon is physiologically and metabolically a good model, especially since atherosclerosis can be induced by appropriate diet. Unlike the rat and dog, it is unsuitable for compulsory smoke exposure; however, there are reports of successful training to smoke voluntarily and inhalation is said to occur. The contractor is required to investigate the training process and to make measurements of smoke intake, inhalation, and retained dose. Ten baboons will be trained and observed over a period of six months. Factors to be measured include number and volume of puffs, blood carboxyhemoglobin as an indicator of inhalation, and physiological response including any development of nicotine dependency. The work includes design of apparatus for presenting, controlling and measuring cigarette smoke.

Screening Less-Hazardous Cigarettes: Acute Bronchitis as an Indicator. Screening tests for less-hazardous cigarettes should relate to smokers as closely as possible. The use of volunteers is scientifically attractive, especially if a rapid, sensitive test is available. Closing volume measurement is a leading candidate since it is a highly sensitive indicator of early response of the small airways to tobacco smoke. The contractor is required to study this, but not to the exclusion of other candidate tests.

The work will depend on volunteer novice smokers because long-term smokers are likely to have chronic lung changes impairing sensitivity. The contractor will evaluate closing volume measurement, recommend procedures for routine use, and recommend other tests if appropriate.

If the test comes into routine use it will offer opportunity for extremely valuable prospective studies of progressive respiratory impairment in smokers, through following selected cohorts; this would also validate early indicators as predictors of long term effects. The contractor is required to make a critical appraisal of this.

Metabolic Studies on Tobacco Smoke Constituents. The contractor is required to study metabolic pathways of four tobacco smoke constituents in

experimental animals: carbon monoxide, hydrogen cyanide, nitrogen dioxide, and nicotine. The RFP emphasizes that this is not to be a basic research program in metabolic biochemistry or toxicology/pharmacology of chemical activity. It is to supplement the scanty existing knowledge of pathways and chemical changes in exposures at smoking concentration levels, using uncomplicated single compounds or simple mixtures. The contractor will review the literature, expose animals by the respiratory route, and follow significant pathways. The controlling rates of transport and chemical change are to be determined. The work will be done with the compounds singly, in combination, and added to mild cigarette smoke. Results will be useful in four fields: identification of high-risk individuals, protection of high-risk individuals, and development of drugs to assist withdrawal or to protect the smoker.

Standardization of Aryl Hydrocarbon Hydroxylase Assay, as a Screening Method to Determine Smoking Hazards in Man. The vigorously active field of enzymes in chemical carcinogenesis suggests a promising approach to identification of high-risk smokers. Aryl hydrocarbon hydroxylase (AHH) is believed to have an important role in potentiation of carcinogens via epoxide formation. AHH is inducible by carcinogenic hydrocarbons and there is a positive correlation of high inducibility with pulmonary carcinoma.

Laboratory assays for AHH exist but they are unsuitable for large-scale field application. The present project anticipates the need for a routine clinical test. The contractor is required to investigate the feasibility of a screening test and to recommend, if practical, a routine procedure for future full-scale development and standardization.

Although the project has this strictly practical and limited objective, it is also likely to point the way to more expeditious laboratory procedures in basic research.

Clinical Trials for Pharmacological Approaches in Smoking Withdrawal. An explicit objective of the Smoking and Health Program is to help those who want to stop smoking. This project will test two groups of known drugs, both related to the role of nicotine in addiction. Nicotine agonists lower the threshold of acceptance (e.g. lobeline, cotinine, and nicotine itself); nicotine antagonists block its physiological effects (e.g. benactyzine, chlorpromazine, propanolol). The contractor will test five of each type. The subjects will be volunteers, their initial response to tobacco smoke will be measured, and randomly selected groups will receive standardized counseling and one of the drugs or a placebo for six weeks. Case-histories will be followed for one year.

Evaluation of the Acceptability to Smokers of Candidate Less-Hazardous Cigarettes. Experimental less-hazardous cigarettes are being tested in large-scale bioassays this year. As these and other experiments develop specifications to minimize carcinogenicity and other health hazards, candidate cigarette models will emerge. It will be necessary to determine whether these will be acceptable to smokers, but no routine method or service for such testing exists.

The contractor is required to identify the best criteria of acceptability, develop procedures for a routine evaluation service, test them in a pilot run, and finally demonstrate the proposed service in comparative evaluation of ten cigarette types.

If the demonstration is satisfactory the NCI expects to negotiate for future routine services in support of the less-hazardous cigarette program.

RFPs To Be Written

In addition to the nine new contracts described above, there are four other projects for which a need has been established and contractual effort planned for the 1974-75 period. These are described below.

Prospective Epidemiological Study. This is a large-scale prospective epidemiological investigation to complement the retrospective studies now being carried out by the American Health Foundation (described previously) and go beyond the scope of that study in several important areas. It would have a statistically adequate stratified sample of the population which would provide for data on demographic sectors such as age, race, sex, economic status and geographic distribution. It would also be extended to provide sample groups in various occupational classes and in different classes and degrees of urban pollution. With regard to occupational groups, Selikoff has reported that asbestos workers who smoke are at much higher risk of lung cancer than those who do not; the same is probably true of uranium miners. Surveys should certainly be extended to other occupational groups. Urban pollution is probably significant because both CO and NO_x are positively correlated with increased incidence of cardiovascular problems, and smokers receive significantly larger daily exposure to these gases than nonsmokers in the same area. The prospective study would investigate the neoplastic and non-neoplastic pulmonary and cardiovascular effects of different types of cigarettes (e.g. filter vs non-filter, types of filter, high vs low tar and nicotine) in the population segments described.

Dosimetry in Inhalation Bioassays. A research effort in dosimetry as it applies to the inhalation bioassay models is highly desirable. At the present time it is possible to generate and measure a presented dose; that is,

the concentration of smoke or a smoke component to which the animal is exposed. The amount of this dose that the animal actually inhales can be estimated but not with the desired accuracy. The dose which the animal retains cannot be measured except by radioactive tracers, and it is not known whether the tracer simulates tobacco smoke generally or only some portion of it. The sites of retention of particles of different sizes, or of various gaseous components, and the subsequent migration of these components under the influence of clearance mechanisms is not adequately known. Techniques to maximize retained dose are also needed. Full evaluation and understanding of inhalation bioassay data will require knowledge of these phenomena, and attainment of this knowledge is an applied research task well within current technology.

Cigarette Combustion. There is no question that the combustion properties of cigarettes influence the tumorigenicity of smoke condensates. It is planned to conduct a study of the combustion process in cigarettes, identify the cigarette parameters which control combustion, and vary combustion and classify the resulting products. The end-product will be a computer simulation model which can be used to guide the design of less hazardous cigarettes.

Sebaceous Gland Bioassay. It is planned to assay cigarette condensates from the First and Second Cigarette Experiments by the sebaceous gland test. This test is much more rapid than mouse skin painting bioassays, and preliminary work suggests the results are comparable. When the sebaceous gland test data are available, it will be possible to compare some 40 cigarettes assayed by five methods: mouse skin painting, sebaceous gland, macrophage inhibition, ciliatoxicity and cytotoxicity. ECI will do the statistical analysis and evaluation.

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July 1, 1973 - June 30, 1974

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SUMMARY REPORT
OFFICE OF THE ASSOCIATE DIRECTOR FOR FIELD STUDIES & STATISTICS
DIVISION OF CANCER CAUSE AND PREVENTION
July 1, 1973 - June 30, 1974

Introduction

The prevention of cancer is the major goal of the Field Studies and Statistics Area. We attempt to do this by uncovering the determinants of human cancer (i.e., epidemiology) and by providing data and consultation for other scientific workers, many of whom share our goal. At the Spring 1974 Science Writers Seminar of the American Cancer Society, a speaker estimated that 40% of cancers in women and 20-30% of cancers in men were direct results of diet. Work by NCI on cancers among migrants, particularly among Japanese migrating to Hawaii, plus earlier data on breast cancer, led to these estimates. In attempting to prevent cancer, studies of childhood cancer give the potential for the early discovery of environmental cancer agents, such as radiation, which are controllable and we have a strong effort in this direction.

The implementation of the data we develop is in the hands of other people, often the Cancer Control program, the Food and Drug Administration, EPA, etc., and we have been successful in the past year in setting up good working relations with the appropriate people. We look to payoff in the future.

The professional staff of the Field Studies Area look upon themselves as the "jaundiced eye" or balance wheel of the Cancer Program examining new reports, testing proposed etiologies, evaluating claims for new treatments, or new "causes" for the many forms of cancer. Much of our work is directed toward diminishing the cognative dissonance of enthusiasts or special pleaders in the cancer research (and treatment) fields.

Accomplishments

Several important findings were made this year:

1. Colon cancer - at least in Japanese migrants, and probably in all populations is related to the "western" diet -- high intake of beef, fats, legumes and starches.

2. Stomach cancer - may be initiated very early in life -- before the age of 15, perhaps.

3. Stomach cancer in coal miners does not appear to be a result of the coal mining per se, but probably is associated with a generalized social class factor. It is a low social class disease.

4. There is a cluster of excessive brain cancers in Eastern Kentucky, probably associated with an as yet unknown environmental pollutant.

5. Kidney transplant patients (immune suppressed) show a large increase in reticulum cell sarcomas and possibly some increase in other forms of cancer.

Data on both incidence and mortality were published in considerable detail:

1. The report of the Third National Cancer Survey was published, for all three years of the survey, and by survey area.

2. Cancer mortality data, by county, by site, by sex and race for the 20-year period 1950-1969 were published, giving total numbers of cases, and rates. This will be followed by maps, permitting visual identification of clusters.

Administrative Relations and Problems

We are pleased that the newly appointed Deputy Director is an epidemiologist. The concern expressed last year that there is no member of the Cancer Board who is an epidemiologist or a biostatistician, remains. Last year we said "...it [is] distressing that there is no one on the Board who devotes his professional life to the direct prevention of human cancer." This is still true.

Our relationships with organ site task forces remain as they were before. We provide expertise and liaison upon request. We continue to conduct and support epidemiologic work in areas in which we have special talents -- childhood cancer, familial and genetic cancers, migrant studies, international cooperative studies, certain sites, such as digestive system. We urge that the task forces be charged with supporting other important research. We have begun to undertake human environmental cancer studies. We are working on an inter-agency agreement with EPA and NIOSH with respect to studies of hazardous chemicals in the environment.

The major problem we face derives from other activities of the Cancer Institute. Our statistical and ADP consultants are being called upon to provide support for large scale contract programs, which, in their development did not include statistical or ADP expertise. Most contractors do not have adequate statistical staffs. We cannot meet all the needs generated by NCI research contracts, more attention in the awarding of research contracts should be given to the ability of the contractors to perceive their statistical and data handling needs, and to hire statisticians and allied specialists to service contract supported research.

We anticipate increased work with the Cancer Control area. They, too are very short of statistical expertise. With current staff levels we will not be able to provide the planning they need in order to set up appropriate evaluation systems for their programs. Within our own program we will be extremely hard pressed to provide competent project officers to monitor any substantially increased number of contracts. Recent administrative rulings restricting project officers from being members of technical advisory groups contribute to our problems.

We have added an air pollution, environmental cancer specialist, through the Federal-State exchange. Work is developing in many areas in the epidemiology of environmentally-related human cancer. We will assign additional personnel to this work as slots become available.

The first tentative steps toward cooperative work with Soviet Russia in cancer epidemiology were taken at a conference in Erevan, Armenian SSR in January 1974. U.S. data on incidence, mortality and survival, plus some special data on skin cancer have been sent to Soviet Russia. Two American physician-epidemiologists have indicated their interests in becoming exchange fellows with the Russians. Some coordination with the cooperative group working on environmental and industrial hazards will be necessary. Steps for our cooperation with the American representatives of this group have already been taken.

A major segment of the Biometry Branch, the Mathematical Statistics and Applied Mathematics Section, has been moved off the NIH campus and into the Landow Building. It is still to early to see if this will affect the consultation done by the professional staff of this Branch.

Other Major Activities

International:

Japan - The Atomic Bomb Casualty Commission contract has been in force for over a year. From it we have seen the continued accelerated incidence of cancer in persons exposed to ionizing radiation in Hiroshima and Nagasaki. The contract has provided a basis for training of Japanese epidemiologists. This activity has been very successful, and the Japanese Government, for the first time, will contribute substantial funds for the continuation of this research activity in FY 1975. The data on Japanese migrants to Hawaii continue to support the hypothesis that the "western" diet predisposes to colon cancer. Work on other sites (breast, lung, prostate) has not proceeded far enough to give firm results. The radiation-cancer data from Japan are being supplemented by studies in Boston showing a very high incidence of second primaries in cancer patients treated with high doses of radiation. Further work with the Japanese on environmental mutagenesis and carcinogenesis has resulted from the U.S.-Japan cooperative medical-scientific program.

International Agency for Research on Cancer (IARC) - The field studies in Iran on cancer of the esophagus have progressed very well. Preliminary results are consistent with a multi-factorial hypothesis consisting of:

(a) the presence of a carcinogen in the food (quite possibly nitrosamines), combined with

(b) possible dietary deficiency, most likely Vitamins A, B_2, and C.

Plans have been developed for appropriate next steps to take to confirm (or deny) this hypothesis. Many other factors have been eliminated, including the use of drugs, hot tea drinking, and dyes used in rug making.

The studies on Burkitt's lymphoma in Uganda may well have come to an end as a result of the March-April killing of many Lugbara people (the major population group in the West Nile district, where our studies were being done) following a military uprising against General Amin.

The third volume of data on carcinogens, including human epidemiology data was completed, with FS&S staff people participating. A first conference on environmental and industrial carcinogenesis seeking to exploit the excellent follow-up and personal identification systems of several of the European countries, was held in Lyon.

International Union Against Cancer (UICC)

Under the aegis of the UICC, the Epidemiology Branch is collecting data for an international comparison of pediatric cancer around the world. A monthly newsletter is used to communicate important observations on the origins of childhood cancer to pediatric oncologists throughout the world.

The international registry of epidemiology and epidemiologists (as a part of the International Cancer Data Bank) is about to begin operation as a cooperative activity of UICC and NCI.

Other International Activities

Migrant studies involving Colombia have clarified the role of intestinal metaplasia of the gastric mucosa as a potential precursor lesion. Cooperation with other South and Central American countries is being explored through the extension of the work to Costa Rica, and through a possible contract with the Pan American Health Organization to study bladder cancer, as a first attempt at cooperative work with Latin American countries.

Domestic Activities

The special interests we have in familial and genetic cancers and in childhood cancers continues. The familial cancers (e.g. medullary carcinoma of the thyroid and pheochromocytoma) give an opportunity for effective secondary prevention through the frequent examination of family members who are at high risk.

Childhood cancers are being pursued through a national registry of death certificates, a followup of children born in military hospitals where data exist on their intra-uterine exposure to x-irradiation, and in cooperation with the Third National Cancer Survey which permits study of incidence as well as mortality.

Particular attention has been paid to the survival of black children with acute lymphocytic leukemia. As for most forms of cancer in blacks, survival is lower in blacks than in whites.

The National Wilm's Tumor Survey has confirmed the high rates of aniridia, and hemihypertrophy in Wilm's tumor children. These are in addition to previously observed high frequency of genito-urinary defects and familial incidence.

With respect to adult cancers, two avenues are being followed in an attempt to exploit the very large case collections in the VA hospital system.

One avenue involves developing epidemiologic studies under VA direction for certain sites of special interest, such as pancreas, liver, esophagus, prostate, bladder. This work is moving very slowly. The avenue seems to be full of potholes. The other approach is through a contract with the Followup Agency of the NAS-NRC. Patients with specific medical procedures, or diagnosis, will be evaluated for unusual cancer incidence or mortality (e.g. tropical sprue and lymphoma). Current work includes a followup of Korean war veterans who were given adenovirus vaccine contaminated with SV-40, among other studies.

Other major areas of work involve studies of prognostic factors, particularly in breast cancer, and Hodgkin's disease. An emphasis on the characteristics of patients who might benefit from certain forms of therapies, and the characteristics of those who might not was developed out of the cooperative studies of cancer of the prostate, undertaken with the Veterans Administration. These studies helped physicians tailor their treatments to their prostate cancer patients. The concept of the "Epidemiology of Treatment" arose out of these studies and it should develop into a fruitful new field of study and research.

One other area of importance is the study of animal cancers in a partly natural environment. The Veterinary Data Program continues to provide a basis for study of cancers in different breeds, strains, species, and to provide a test for theories of transmission of cancer.

Major Data Publications

Two major sets of data were published in the past:

1. A summary of cancer mortality over the twenty year period 1950-1969, by county, for every one of the over 3000 counties in the United States. These data were presented for each sex, for two racial groups (white and others), for 30 plus sites.

2. The full three year report of the Third National Cancer Survey, including total U.S. data (for approximately a 10% sample of the U.S. population), and data for each of the several survey areas separately.

These two sets of data have proved very useful in helping answer many of the questions which have risen out of the Conquest of Cancer Program. Some special spin-offs were developed to reply to problems faced by other government agencies. For example, data on the incidence of skin cancer (in four of the TNCS areas), which showed a doubling of skin cancer for each 8 to 10 degrees of latitude, proved to be very important to the Department of Transportation in its considerations of the Supersonic Transport.

Major Program Thrusts

The Third National Cancer Survey and the End Results Program have given way to the SEER (Surveillance, Epidemiology and End Results) Program. Through this program, we hope to develop early data on incidence, mortality and survival, on a grass-roots, "out there" level. Through SEER we hope to increase the supply of epidemiologists, by having them cut their intellectual

teeth on real problems arising in real settings in the field. Because of the shortage of chronic disease epidemiologists, we are limited in the problems we can handle well. The teaching aspects of SEER should help overcome this. We look to special studies in some of the SEER areas to help explain why blacks, almost uniformly, have poorer survival than whites for the same forms of cancer. We look to studies in other areas to uncover what it is about American Indians, or Chinese, or Japanese that leads to their having different mortality patterns from cancer than the whites in the same areas. We look to an extension of the SEER concept to help the managers of the Cancer Control Program evaluate the effectiveness of their activities.

Two other major conceptual areas are being worked on. The first, environmental cancer (in the sense of the man-made additions to our environment) has begun to gather some momentum, in large part because of our addition of a staff member wholly concerned with these problems. We have had collaboration with EPA, NIOSH, and FDA among other government agencies. The other area, the elucidation of the social and psychological components of cancer etiology has not yet evolved into a program, although several research proposals have been made.

Plans have been developed for a conference and a monograph on high risk groups, that should provide useful information to screeners and to the Control Program. The conference will be held in December 1974, with the cooperation of the American Cancer Society.

Summary and Prospects

A great deal of work remains to be done. There are too few people to do it all. We do not have enough staff to monitor many additional contracts. New data have been developed, and the prospects for better "grass-roots" work in epidemiology are good. Our cooperation and collaboration with other government agencies, with international agencies and with foreign governments has improved substantially. We have lost one senior person through early retirement, in part because of federal pay ceilings. We are likely to lose more. Some promising young staff are developing, who, in a few years, should be able to make up these losses.

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Carcinogenesis Program: Second Annual Collaborative Conference, November 25-29, 1973, San Antonio, Texas.

Consultant:

Carcinogenesis - Bioassay Segment on design and analysis of experiment
Carcinogenesis - Information Segment on an epidemiological study of N-nitroso compounds
EPA - Analysis of carcinogenicity experiment with dieldrin

Lectures:

WNAR (Western North American Region) of the Biometric Society, June 17-18, 1974 on "Protocol Design and Approaches to Survivorship with Censoring."

Bernard H. Fox, Ph.D.

Conferences:

Conference on Public Education. Cancer Control Program, NCI. July 31-Aug.3, 1973. Session Moderator: Roles of Health Professionals in Determining Public Attitudes and in Promoting Compliance with Health Care Practices.

World Population Society, First Annual Conference. Chairman, Paper Session: Influencing Population Trends (Motivational), Feb. 9, 1974.

Consultant:

National Eye Institute, Office of Biometry and Epidemiology
National Institute on Alcohol Abuse and Alcoholism, Associate Director for Program Development and Evaluation

HONORS AND AWARDS (Cont.)

Cancer Control Program, Deputy Director
Cancer Control Program, Education and Training Branch
National Institute of Neurological Diseases and Stroke, Perinatal Research Branch

Committee Membership:

Cancer Control Education Review Committee, CCP
Cancer Control Program, Ad Hoc Committee on Unsolicited Proposals
Rehabilitation Branch, CCP, Ad Hoc Review Committee
Rehabilitation Branch, CCP, Ad Hoc Committee on Unsolicited Proposals
Education and Training Branch, CCP, Ad Hoc Review Committee
Education and Training Branch, CCP, Ad Hoc Committee to Plan a Working Conference on Cancer Public Education
National Institute of Mental Health, Extramural Technical Review Cmte.
International Committee on Alcohol and Traffic Safety
Committee on Alcohol and Drugs, National Safety Council
Committee TO-14, Driving Simulation, Highway Research Board, National Academy of Sciences-National Research Council
Conference on Injury Control and Emergency Health Services (APHA)

John R. Goldsmith, M.D.

Conferences:

Satellite Symposium on Health Effects of Air Pollution, Tokyo, Japan, September 29, 1973.

Western Industrial Health Conference on the California Plans for Occupational Safety and Health (Lecturer), October 5, 1973.

Ocean Pollution Workshop - Study to assess potential effects of man-generated substances and activities on the marine environment, Woods Hole, Mass, October 29, 1973. (Report to be published by the NAS.)

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HONORS AND AWARDS (Cont.)

California Thoracic Society, Annual Scientific Meeting, April 4-6, 1974.

Consultant:

California Department of Health - Scientific Basis for Emergency Plans on Air Pollution
Electrical Power Research Institute - Impact of Power Generation on Environmental Health
National Academy of Sciences - Study on the Health Effects of Air Pollution
U.S. Senate Committee on Public Works - Controls on Lead Emissions from Automobiles and the Presence of Lead in Gasoline

Lectures:

Yale University, New Haven, Connecticut, March 14, 1974, "Health Effects of CO in Relation to Emission Control Policy."

Editorial Committee:

Editorial Review for Archives of Environmental Health

Associate Editor:

Panel, National Academy of Sciences/NRC "Vapor Phase Organic Air Pollutants from Hydrocarbons."

Committee Membership:

U.S. National Committee on Vital and Health Statistics
Chairman of Consultant Panel, Statistical Data on Environment
National Health Air Conservation Commission
Councillor of American Thoracic Society
Society for Occupational and Environmental Health
Chairman of Air Quality Advisory Committee, California Dept. of Health
Panel on Vapor Phase Organic Matter, NAS/NRC

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Conferences:

NCI-ACS Executive Staff Conference, Hershey, Pa., August 22-23, 1973.
Biological Effects of Ingested Asbestos, Joint NIEHS-EPA Conference, Durham, N.C. November 18-20, 1973.
Annual Princeton Conference on Applied Statistics, Princeton, N.J., December 7, 1973.
NCI Symposium on Combined Modality Therapy of Cancer, Denver, Colorado, February 7-9, 1974.
Symposium on Statistics and the Environment (NAS,ASA), Washington, D.C. March 6-8, 1974.
ACS' 1974 Science Writers Seminar, St. Augustine, Fla., March 22-27, 1974.
American Assn. for Cancer Research Symposium on Environmental Determinants of Human Cancer, Houston, Texas, March 29-30, 1974.

Consultant

Environmental Protection Agency
Food and Drug Administration
Natl. Institute of Occupational Safety and Health

Lectures

Columbia University College of Physicians & Surgeons, New York, March 15, 1974
Millersville State College, Millersville, Pa., April 17, 1974

HONORS AND AWARDS

Associate Editor

Cancer Research
Blood

Committee Membership, etc.

Adjunct Professor, Biostatistics, University of Pittsburgh
Clinical Associate Professor, Medical Statistics, Georgetown University School of Medicine
Fellow, International Statistical Institute
Member, Diagnostic Research Advisory Group, NCI
Member, Council for Analysis and Projection, ACS
Chairman/Executive Secretary of the Committee of Presidents of Statistical Societies (COPSS)
Member, Board of Directors, American Statistical Association
Member, Executive Committee of the Biopharmaceutical Subsection, Biometric Society
Councillor-at-Large of the Society for Occupational and Environmental Health
Member, Advisory Board, Research Support Center, V.A. Hospital

Serial No. NCI-4402
1. Office of the Associate Director for FS&S,DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Effect of Information Available to the Pathologist Who Reads Slides of Animal Tumors in Carcinogenesis Testing

Previous Serial Number: None

Principal Investigator: Thomas R. Fears, Ph.D. and Bernard H. Fox, Ph.D.

Other Investigators: None

Cooperating Units: Carcinogenesis, DCCP

Man Years:

Total	:	.08
Professional	:	.08
Other	:	.00

Project Description

Objectives:

To determine variation within and between pathologists in reading animal slides with full, partial and no awareness of background and treatment information in the experiment.

Methods Employed:

Appropriate experimental design, without unusual features.

Major Findings:

Significance to Biomedical Research and the Program of the Institute:

We hope to establish which of the above level(s) of information for slide reading achieve(s) maximum accuracy and minimum variation of slide reading in animal carcinogenesis experiments, thus improving their efficiency.

Proposed Course of Project:

Planning is now in progress. An amendment to an existing contract is planned for one phase of this work and an independent contract for the other phases. It should be ready for contract letting in late fall, 1974 and be completed by the end of FY 1975.

Date Initiated: September 1973

Serial No. NCI-4403
1. Office of the Associate Director for FS&S, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Review of Delay Behavior and Attitudes in Cancer Screening, Referral and Treatment

Previous Serial Number: None

Principal Investigator: Bernard H. Fox, Ph.D.

Other Investigators: Phyllis Rosenberg

Cooperating Units: Biometry Branch, FS&S

Man Years:

Total	.08
Professional	.03
Other	.05

Project Description

Objectives:

To collect the bulk of existing information on behavior and attitudes relating to delay in pre-symptomatic cancer screening and post-symptomatic referral and treatment.

Methods Employed:

Literature search and consultation

Major Findings:

Preliminary indications are that the general types and frequencies of reasons for delay andnon-delay are known, but not their distribution in specific race, sex, national and age groups. These reasons seem to be fairly consistent in the literature studied thus far.

Significance to Biomedical Research and the Program of the Institute:

Planning executives at NCI and in organizations elsewhere repeatedly ask what is known about motivation for delay, in order to promote earlier detection and treatment than exists now. This review will provide an organized analysis of relevant information with remarks on probable validity,

consistency, and usefulness for changing behavior. It will also point to missing information and suggest research with greatest payoff potential.

Proposed Course of Project:

Literature collection and consultation have started. A complete outline is planned for early fall with the review itself scheduled for completion by years's end.

Date Initiated: December 1973

Serial No. NCI-4404
1. Office of the Associate Director for FS&S, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Psychiatric Illness and Cancer Risk

Previous Serial Number: None

Principal Investigator: Bernard H. Fox, Ph.D. and Margaret A. Howell, Ph.D.

Other Investigators: None

Cooperating Units: Carcinogenesis, DCCP

Man Years:

Total	.07
Professional	.05
Other	.02

Project Description

Objectives:

To establish the role of schizophrenia and other types of mental illness in reducing or increasing cancer risk.

Methods Employed:

Literature review and evaluation, and epidemiologic analysis of veteran records.

Major Findings:

Several studies have recorded a lower than expected cancer risk among the psychiatrically ill. A biochemical effect, either from the illness or drugs used for treatment, has been suggested as the cause. After review of existing evidence the present investigators hypothesize that relatively low reported mortality due to cancer in psychiatric groups may result (1) from statistical artifacts in the data, including failure to take into account competing risks from other causes of death, incorrect designations of cause of death, faulty diagnoses, both of cancer and of mental illness, biases in the population of patients admitted, and length of stay in hospital; and (2) if the reduced mortality rates are indeed correct,from effects of institutionalization in reducing known environmental hazards and increasing known protective influences.

Significance to Biomedical Research and the Program of the Institute:

If it should be established that a mental disease confers protection, analysis of blood components and therapeutic drugs might identify the factors responsible If it is concluded that neither disease nor drugs confer protection, unneeded research would be avoided.

Proposed Course of Project:

A fairly thorough review of the literature has been made and a hypothesis (see above,findings) submitted for publication. We are exploring with the National Academy of Sciences the possible analysis of mortality causes in VA records of WWII veterans discharged or hospitalized with mental disease. Whether this study is done or not, further avenues of investigation will be examined, since similar reports of deficient mortality rates keep surfacing.

Date Initiated: August 1973

CONTRACT NARRATIVES
OFFICE OF THE ASSOCIATE DIRECTOR
FOR FIELD STUDIES AND STATISTICS
DIVISION OF CANCER CAUSE AND PREVENTION

Fiscal Year 1974

CONFFRENCE ON RELIABILITY AND BIOMETRY (NO1-CP3-3213)

Title: Same

Contractor's Project Director: Dr. Frank Proschan

Project Officer (NCI): Dr. Marvin A. Schneiderman

Objectives: To organize and hold a Conference on Reliability and Biometry with the following goals: (1) to inform biometricians of the problems, models, concepts, and methods of quantitative reliability theory and application, and to inform reliability theoreticians concerning biometry; (2) to stimulate the exchange of ideas between biometricians and reliability theoreticians; (3) to encourage new research in each field as a result if insights, inspiration, and motivation gained from the other field; and (4) to produce a volume of papers presented and summaries of discussions held at the Conference so that the many workers in biometry and quantitative reliability not attending might still benefit from the Conference in subsequent years.

Methods Employed: The Conference was held at the Florida State University, Tallahassee, Florida, July 9-27, 1973. The program was planned to give a good deal of time for informal discussion and exchange of ideas among Conference participants. Two or three papers a day were scheduled; each paper was allowed 50 minutes for presentation, followed by questions and answers and as much discussion as desired.

Significance to NCI Program and Biomedical Research: Thirteen graduate students in biometry were selected to participate in the Conference under the sponsorship of the National Cancer Institute. Some of the benefits to the biometry graduate students attending the Conference under NCI sponsorship are: (1) they received a broad comprehensive over-view of reliability and biometry and their interface; (2) students were helped and guided on specific problems and questions in connection with their own studies and dissertation topics; (3) students were inspired by personal contact with the leading research scientists in reliability and biometry; and (4) they were encouraged to continue their studies and research in biometry and to seek careers in the field.

Date Contract Initiated: January 1, 1973

Current Annual Level: $9,360

CONTRACT NARRATIVE
OFFICE OF THE ASSOCIATE DIRECTOR
FOR FIELD STUDIES AND STATISTICS
DIVISION OF CANCER CAUSE AND PREVENTION

Fiscal Year 1974

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP-43342)

Title: Etiology of Esophageal Cancer in the Caspian Littoral of Iran

Contractor's Project Director: Dr. C. S. Muir

Project Officer (NCI): Dr. Marvin A. Schneiderman

Objectives: To develop a case-control study to estimate the risk for esophageal cancer associated with a number of factors. All patients with cancer of the esophagus in the area covered by the Cancer Registry, that is, the provinces of Mazandaran, Gilan and the district of Ardebil, will be interviewed and compared with their matched controls.

Major Findings: First results indicate a possible combination of dietary insufficiency (Vitamin A, B_2 and C) combined with a carcinogen present in certain foods, as the causal complex.

Significance to Biomedical Research and the Program of the Institute: Data collection and detailed statistical analyses is a long-term process. It is anticipated that leads from these studies may be useful in the prevention of cancer in the United States.

Proposed Course: Work is underway to identify the possible carcinogen present in certain foods.

Additional data collection and detailed statistical analyses remain to be accomplished in the areas of (a) population-sampling for personal, social, and nutritional practices; (b) surveys of household and village customs and practices, as well as agricultural and other economic activities; and (c) analytical chemistry on food samples already collected.

Date Contract Initiated: January 1, 1973

Current Annual Level: $45,000

CONTRACT NARRATIVE
OFFICE OF THE ASSOCIATE DIRECTOR
FOR FIELD STUDIES AND STATISTICS
DIVISION OF CANCER CAUSE AND PREVENTION

Fiscal Year 1974

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO (NO1-CP-33327)

Title: Group Testing for Screening Carcinogens

Contractor's Project Director: Dr. Robert M. Elashoff

Project Officer (NCI): Dr. Thomas R. Fears

Objectives: To develop mathematical techniques based on the theory of group testing to permit more rapid and efficient testing of chemicals for potential carcinogenesis.

Major Findings: Analysis was done of results of pilot experiments, particularly the weight gain and food consumption during the eight week study. There was also an extensive study to determine the consequences of using one or two stage screening designs.

Five manuscripts were prepared for publication on (1) screening for tumorigens using sequential designs, (2) screening using one-stage designs, (3) evaluation of binomially distributed pseudo-random number generators, (4) a review of the modelling of combinations, and (5) the analysis of the dose determination study.

Significance to Biomedical Research and the Program of the Institute: Carcinogenesis testing is a long-term expensive process. With over 20,000 materials to test for carcinogenicity unless more efficient methods are developed testing will take a very long time. This research holds promise for more rapid testing, and for yielding information on interactions of materials.

Proposed Course: Continued close collaboration with groups doing biological testing of the materials to speed the development of mathematical models.

Development of non-sequential group testing schemes (a compromise between financial economy and time economy).

Development of further applications of group testing.

Analysis and monitoring of long-term experiments.

Date Contract Initiated: June 24, 1971

Current Annual Level: $99,269

SUMMARY REPORT
BIOMETRY BRANCH
July 1, 1973 - June 30, 1974

While the manner in which the Biometry Branch carries out its work changes in response to the needs of the National Cancer Institute and the National Cancer Program, its 3 basic functions remain unchanged: a) conduct of research on the etiology of cancer in man; b) provision of statistical consultation and support to other research investigators; c) dissemination of baseline data against which administrators can measure their successes and failures in reducing cancer incidence and mortality. These functions encompass a wide range of scientific and managerial skills in bringing people and data together.

Third National Cancer Survey

Field work for the Third National Cancer Survey, conducted in 7 metropolitan areas, 2 states and Puerto Rico and which registered all new cancer cases diagnosed in the years 1969-1971, has come to an end. The data have been processed and stored on tapes and analysis leading to the preparation of monographs and other publications is well underway. The Demography Section of the Branch has been given the primary responsibility for those analyses to be carried out by the intramural staff. However, we emphasize that the Third National Cancer Survey represents such a rich source of data that investigators from other institutions must be recruited to take on specific research assignments. To assure a well-rounded program of publications a Committee on Utilization has been appointed with representatives from the National Cancer Institute, contractors participating in the Survey, chronic disease epidemiologists and medical care analysts chaired by the Survey director, Dr. Cutler.

The core of basic incidence data for the 3-year period 1969-71 has been prepared and is scheduled to appear as a publication in the NCI monograph series in mid-1974. An advance report of the 1969-71 incidence data has been distributed to NCI staff and principal investigators. A total of 181,000 newly diagnosed cancers were enumerated, yielding an average annual incidence rate of 300 per 100,000 population. The age-adjusted incidence was considerably higher for males than for females, 347 vs. 270 per 100,000 population, and Blacks had higher age-adjusted rates than Whites, 319 vs. 298 per 100,000 population. Blacks had markedly higher rates for prostate, esophagus and lung. Data from the Third National Cancer Survey have been extensively utilized in the new revision of Cancer Rates and Risks. Previous editions have served as a useful reference volume for physicians, medical students, public health workers, cancer educators, American Cancer Society staff and volunteer workers and an extensive backlog of requests for the new edition has already been received. Findings from the special skin cancer survey indicated that the estimated incidence for this site has been grossly understated in past survey and registry reports. The large disparity between the number of cases enumerated and that expected on the basis of previous reports has led to a review of the data by a number of consultants,

including a National Academy of Science committee. While this review has not been completed, we anticipate that our findings on skin cancer incidence will prove to have a firm, factual basis.

SEER Program

Implementation of the National Cancer Program requires information that can be supplied only by a network of cancer registries. The national goal of research and action directed toward prevention, diagnosis and treatment requires information on the nature and magnitude of the total cancer problem and its components to evaluate progress. The SEER program which has initiated new population-based registries in the United States with NCI support is a response to this need. The representation of registries in the SEER program is designed to give wide geographic coverage and to include substantial representation of minority populations (Blacks, Spanish-Americans, American Indians, Japanese, Chinese). The Connecticut and California registries as SEER participants will continue to provide data on cancer incidence and patient survival. Two areas in the Third National Cancer Survey--Detroit, state of Iowa--have been transformed into a continuing registry system and will become important components of the SEER program. The cancer registries in Utah and New Mexico are converting operations so that they will generate incidence data and serve as centers for epidemiologic research. Registries in Seattle, Hawaii and New Orleans are still in the developmental phase but should be fully operational as SEER members by the end of 1974.

The Biometry Branch has been prepared to undertake the national coordination of the SEER network of registries because of its past experience in collaboration with the End Results Group. However, coordination of work directed to cancer epidemiology and more sophisticated studies of prognostic factors envisioned for the population-based cancer study centers and the Cancer Research Centers require new mechanisms to facilitate exchange of information to enable the Centers to develop programs compatible with broad national goals. Plans to create a coordinating committee, including the directors of the several Centers as members, are being developed.

End Results

The End Results Program now emphasizes the collection and analysis of data from population-based registries (SEER program). Access to a larger volume of cases available from population-based registries will permit in the future more detailed examination of the experience of population subgroups. For example, in the past year more intensive studies of the survival experience of Blacks were undertaken. When the disease appears localized to the organ of origin, as in bladder cancer, the difference in survival between Black and White patients is limited to the first few years following diagnosis. For other cancer sites, typified by prostatic cancer, Black patients have continued to display excess mortality for as long as 10 years following diagnosis. Studies of factors including quality of medical care, differences in histologic types and/or immunological reactions which could account for the poor survival experience of Blacks need exploration.

Development of more precise classifications of site-histologic type complexes is a prerequisite for improving the quality of registry data for intra-country and international comparisons of incidence and survival. The Branch continues to collaborate actively with specialists from other disciplines in the design and conduct of such investigations. During the past year the Branch collaborated with the Laboratory of Pathology in evaluating the reliability of diagnosis of histologic subtypes of Hodgkin's disease through use of the Rye classification by 16 pathologists from various countries.

The existing U.S. data resources will permit the National Cancer Institute to play a leading role in international collaborative studies of patient survival. Several topics worthy of investigation had been defined in an earlier collaboration with registries in 5 other countries and summarized in NCI Monograph 15. We have initiated conversations on resumption of work with registries in Norway, Finland, United Kingdom and selected other countries will be invited to participate. It has been agreed that the initial comparative studies will focus on cancers of the large bowel and breast.

Clinical and Diagnostic Trials

The development of activities by the Section of Clinical and Diagnostic Trials established in the preceding year continues according to plan. The Section continues to provide the Veterans Administration Cooperative Urological Research Group with a complete spectrum of supporting statistical services. In return the Section has access to the largest set of data available on the course of prostatic carcinoma, and this activity also provides the Section with a substantial background of valuable experience in protocol design, data recording, collection and editing and analyses of data from clinical trials. The studies are conceived to be a self-generating sequential series of investigations continuing over a 15-year period. This relationship provides a suitable environment for defining numerous general methodological studies appropriate to the analysis of data from clinical and diagnostic trials.

The Section also provides consultant services for several large-scale clinical trials to clinicians and scientists in the National Cancer Institute and other institutions.

Migrant Studies

The Branch's study of migrant populations oriented to changes in site-specific cancer risks remains focused on migrants from Japan to Hawaii and on intra-country migration in Colombia. The collective evidence continues to support hypotheses on a dominant role for environmental factors, but the importance of the timing of these exposures varies by site. Exposure to events in early life continues to dominate the epidemiology of stomach cancer. On the other hand the sustained rise in colon cancer among Japanese migrants to Hawaii strongly suggests that exposures in adult life after arrival in the United States has modified the risks for large bowel cancer.

The combined epidemiology-pathology studies among Hawaiian Japanese and work in Colombia has helped to clarify the role of intestinal metaplasia of the gastric mucosa as a potential precursor lesion. Review of stomach cancers among Japanese in Hawaii and Japan has revealed different slopes for the age-incidence curves for intestinal- and diffuse-type gastric carcinomas. The curves for intestinal-type among native and migrant Japanese retain the same age slopes, but the curve for Hawaiian Japanese is displaced to the right. Such observations are helpful in development of hypotheses on pathogenesis.

A case-control study in the Hawaiian Japanese--a population undergoing displacement from low to high colon cancer risk and in transition from Japanese- to Western-style diet uncovered for the first time important correlations between food items and bowel cancer. The major findings included:

a) More bowel cancer patients than controls had abandoned the practice of eating at least 1 Japanese-style meal daily.

b) Patients ate meat, legumes and starch more frequently, and the largest patient-control disparity was noted for meat and legumes.

c) Beef and string beans were identified as major contributors to the meat and legume effects. The associations with beef and string beans were enhanced in a subset of patients whose tumors were located in the sigmoid and adjacent colon.

Consistencies between this study and other evidence yielded by international comparisons of populations at high- and low-risk to this disease are apparent, which suggest that the findings on beef have most immediate relevance. The major significance of this study is that the findings may narrow the area of search for etiologic factors and assist in the planning of research on the complex sequence of events leading to bowel cancer. Recent studies on intestinal polyps in high- and low-risk populations for large bowel cancer continue to reinforce impressions on concomitant population variations in intestinal polyps and large bowel cancer. Stemmermann and Yatani have described the increased prevalence and change in anatomical distribution of adenomatous and hyperplastic polyps that has accompanied a marked rise in colon cancer risks among the Japanese migrating to Hawaii.

One point which has emerged with increasing clarity is that the etiology of large bowel cancer is unlikely to be resolved by work conducted within a single population and that progress depends on the broad perspective afforded by comparative population studies needed to generate etiological hypotheses. Large bowel cancer is probably not unique and clarification of the epidemiology of several cancer sites for which etiologic relationships remain obscure may come from comparative studies of sedentes and migrants.

Consultation

The Mathematical Statistics and Applied Mathematics and the ADP Management Sections continue to provide a broad range of consultation in experimental design and systems analysis to scientists in all divisions of NCI and their collaborators supported by research contracts. While these functions have been effectively discharged during the past year, some changes in the nature of problems receiving consultation deserve mention. The conventional type of statistical consultation concerned with the analysis of data from a single designed experiment is now encountered less frequently. There is now much greater demand for consultation on statistical and data handling aspects of large research-contract supported programs for which NCI staff serve as project officers. The need for this type of consultation is straining the Branch resources. It is quite evident that the demand for these expert services has increased in the absence of adequate statistical consultation facilities in the contractors' staffs. The relatively small NCI group cannot meet present and future needs generated by NCI research contracts and more emphasis in the award of research contracts should be placed on the ability of the contractors to hire statisticians and allied specialists to service contract-supported work. Despite these outside pressures of statistical consultation the Sections have continued to be productive in the development of new and improved techniques of experimental design and analytical techniques suitable for the investigation of problems of carcinogenesis.

Problems

The biggest problem in recent years confronting the Biometry Branch has been to develop and monitor its own contract-supported research (Third National Cancer Survey, SEER program, migrant studies) with an intramural staff of smaller than optimal size. These pressures continue with the deployment of staff to the analysis of data collected in the Third National Cancer Survey, while simultaneously developing an expanded network of population-based cancer registries. The ultimate solution must come from decentralization of much of the ongoing analytical epidemiology activities to the population-based study centers. The latter, in turn, depends on expanding the pool of epidemiologists and biostatisticians interested in cancer epidemiology to staff the study centers. The national pool of manpower engaged in these activities is small and totally incommensurate with program needs. A vigorous program of training is urgently needed and the opportunities for training presented within the framework of the SEER program and population-based cancer study centers should be used to advantage for this purpose.

Despite these caveats we can be optimistic about the future. The migrant studies are developing specific etiological hypotheses for gastrointestinal cancers and the findings from the Japanese migrant studies on large bowel cancer continue to play a key role in the planning of activities supported by the NCI Colon Cancer Segment and the National Large Bowel Cancer Project. The Third National Cancer Survey provides data useful for epidemiological studies and the planning and evaluation of cancer control programs. The End Results studies are identifying promising leads on prognostic factors for breast and other cancer sites.

Serial No. NCI-4250
1. Biometry Branch, OADFS&S
   Division of Cancer Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Policy Planning and Liaison with Other Organizations

Previous Serial Number: Same

Principal Investigators: William Haenszel, Sidney Cutler, and Nathan Mantel

Other Investigators: James Murray, David Levin, Charles Brown

Man Years:

Total:	4
Professional:	2
Other:	2

Project Description

Objectives:

The principle objectives are to provide general planning and direction for the scientific program of the Biometry Branch, coordination and collaboration with other major program components of the National Cancer Institute, and active liaison with other national and international organizations.

Methods Employed:

Standard methods of program planning, management, demography, biometry and epidemiology are applied to coordinate the Branch activities and conduct the research projects.

Major Findings:

Development of the National Cancer Program has reinforced the need for an informational network within the United States, with links to international sources of information on cancer. A network of population-based cancer reporting systems providing information on a total population of more than 20 million people has been organized. This network is the foundation of the Program of Cancer Surveillance, Epidemiology, and End Results Reporting (SEER) that is being coordinated by the Biometry Branch. The SEER Program provides a base for a comprehensive cancer data program for the United States, including input from cancer centers, and providing data both for planning and

evaluating cancer control activities. In the development of the SEER Program, particular attention is being given to the collection of descriptive information of clinical interest, e.g., referral patterns, diagnostic procedures in assessing the neoplasm, extent of disease, treatment, and end results. The availability of information on all residents of defined geographic areas will provide data for comparision of different regions and population groups, and will provide a basis for comparing the experience of cancer centers and other specialty institutions with the experience of "the general run of patients". A working committee has been organized to promote compatibility of information from various sources. The guidelines being developed for information exchange within the United States should be very helpful in developing guidelines for exchange of compatible data on cancer on an international level.

Collaboration with various national and international bodies concerned with cancer reporting systems and cancer data has been an important element in Dr. Cutler's activities. He is a member of the Executive Committee of the American Joint Committee on Cancer Staging and End Results Reporting and a member of a WHO working group on international guidelines for End Results Reporting. He is coordinating the contributions of USA cancer registries to the 3rd edition of Cancer in Five Continents to be published by the International Agency for Research on Cancer. Dr. Cutler has participated in several workshops of the Association of American Cancer Institutes on exchange of information and end results reporting.

A particular effort is being made to develop information that will be useful in planning and evaluating cancer control activities. Discussions have been held with Drs. Fink and Jay, Director and Deputy Director of the Cancer Control program. Consideration is being given to concentrating a variety of cancer control projects in several areas with established community-based cancer reporting systems sponsored by the SEER Program in order to facilitate evaluation of the effect of control activities.

Specific proposals are being developed for consideration by the directors of Cancer Centers sponsored by the National Cancer Institute. These proposals are designed to facilitate the collection of data on cancer patients needed by the individual centers, to provide for the exchange of comparable information among centers, and to provide information needed by the National Cancer Institute. The reporting and data processing techniques developed for the SEER Program provide a solid foundation on which to build a data system to meet the needs of the Cancer Centers.

In addition to providing data for a variety of purposes the SEER Program is designed to provide leads for epidemiologic research and an operational framework for implementing ad hoc studies and training programs. Exchange of information and ideas among the participants in the SEER Program is an important element in stimulating ideas and promoting collaboration. To encourage contacts the participants are being organized into a "group" with a chairman and several standing committees. One of these committees will deal with Epidemiology and will include investigators from outside the SEER Program. This should broaden the range of talent and ideas.

The end of the data collection phase of the Third National Cancer Survey, coupled with initiation of the SEER Program which consolidates the collection of incidence and survival data, has led to some realignments in Branch organization. A Field Liaison Section headed by Mr. Geller was created to centralize the channels of communication with the groups participating in the SEER Program. The Demography and End Results Sections headed by Drs. Young and Myers respectively are now concerned primarily with data analysis. The SEER Program has substantially upgraded the responsibilities of project officers for the several contracts. The responsibilities are viewed as primarily scientific, rather than managerial, in nature and a deliberate policy of giving all professional staff, including junior members, project officer assignments has been followed. These duties will grow in succeeding years with development of the National Cancer Program and this step is necessary to develop staff capabilities.

Several staff members are actively involved as members of site-oriented task forces. Mr. Haenszel serves as a member of the Epidemiology Committee of the Breast Cancer Task Force and as a member of the Working Cadre of the National Large Bowel Cancer Project. Dr. Cutler is a member of the Steering Committee of the Breast Cancer Task Force. Dr. Byar is a cadre member of the National Prostate Cancer Project and a member of the Diagnosis and Treatment Committee of the Breast Cancer Task Force.

The collection and processing of data in the Third National Cancer Survey, covering the 3-year period, 1969-71, is complete. Attention is now being given to analysis and dissemination of the available information. An advanced report for the total survey and for each of the nine areas has been prepared. Thus, the basic data on cancer incidence in the United States is being given prompt distribution. A detailed monograph on the incidence of cancer, in which particular attention is being given to the histologic characteristics of cancers originating in each primary site, is in preparation and should be in the hands of the printer about July 1.

The survey data include information which probably has never previously been collected on such a large scale. For example, on a 10-percent sample of patients, information was collected on duration of hospitalization, repeat hospitalizations, treatment received, cost of hospital care, and sources of payment. To insure the fullest utilization of the wide range of information collected, an advisory committee has been appointed. This committee will provide guidance to analytic approaches and to the recruitment of specialized experts to work with the staff on the analysis of the data. A series of papers on individual sites, groups of sites, and selected subjects will be published in individual journals. It is anticipated that these reports will be brought together later in monograph form. Thus, the publication of the voluminous basic data will be supplemented by a series of interpretive analyses on individual subjects.

Dr. Levin has worked on analysis of the Third National Cancer Survey patient interview data and on the SEER Program. He has worked on problems of design of clinical trials, epidemiology of pancreatic cancer, environmental factors in the etiology of cancer, and the role of PVC as a possible carcinogen. He is coordinating a revision of the booklet "Cancer Facts and Figures".

Dr. Levin is a member of the Medical Oncology Contract Review Committee and the Interagency Collaborative Group on Environmental Carcinogenesis; he has worked with several other areas within NIH including the Special Virus Cancer Program's Immunology-Epidemiology section and researchers from the Medicine Branch of NCI. He has served on the mathematics faculty of the NIH Federal City College Upward Mobility Program.

Mr. Mantel's collaborative work with other investigators at the National Cancer Institute has included important consultation with Dr. Goldin in the Chemotherapy area, Drs. Miller, Fraumeni and Priester in the Epidemiology Branch, and Dr. Boone in the Viral Biology Branch. He also continued as a Cancer Research Editorial Advisory Board Member. A subject of dominant interest during the past year has been identification and evaluation of potential environmental carcinogens and in this area Mr. Mantel has been involved in work by investigators at a number of organizations, including F.D.A. (Friedman, Gross, Anello), E.P.A. (Prival, Kolojeski, Lyle), N.C.T.R. (Gaylor), N.I.E.H.S. (Hoel), N.Y.U. Medical Center (Albert, Altschuler). Relative to disease-clustering problems he has worked with Holmes and Flynt at the Center for Disease Control and with Goad at the Los Alamos Scientific Laboratories, and he has provided advice on epidemiologic methods to Drs. Beebe and Jablon at the National Academy of Science and Dr. Landau of the Bureau of Radiological Health, F.D.A. Development of statistical methodology and theory strongly related to the subject matter of his consulting activities has remained during the past year an important part of Mr. Mantel's professional activities and in some of this developmental work Mr. Mantel has been joined by Dr. Brown. The development of statistical methodology has been Dr. Brown's primary concern in which he has collaborated with other members of the Biometry Branch (Muenz, Byar, Hankey) and he has also consulted with the U.S.D.A. (Crittenden), worked on aspects of the SEER Program, and studied prognostic factors in survival of stomach cancer patients.

Significance to the Program of the Institute:

The development of data resources and the provision of statistical consultation services are necessary contributions to the National Cancer Program.

Proposed Course of Project:

Planning efforts will be directed toward development of an optimum mixture of data resources. Consideration is being given to supplementing the SEER Program with a program of sampling from an enlarged geographic base in order to more effectively provide information needed for planning and evaluating the Cancer Control Program and the International Cancer Research Data Bank Program.

In order to more effectively carry out the expanded work scope of the Biometry Branch Program, the staff has been regrouped as follows:

Office of the Chief
Clinical Trials Section
Mathematical Statistics and Applied Mathematics Section
Automated Data Processing Section
Demography Section
End Results Section
Field Liaison Section

The latter three sections are jointly responsible for implementing the SEER Program, under the direction of Dr. Cutler, Associate Branch Chief. The Automated Data Processing Section is intimately involved in helping the participants in the SEER Program organize or reorganize their data processing procedures to facilitate optimum utilization of the collected information.

Honors and Awards

Cutler, S.:

Member, American Joint Committee on Cancer Staging and End Results Reporting

General Secretary, International Association of Cancer Registries

Adjunct Professor, Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh

Haenszel, W.:

Member, American Cancer Society Advisory Committee on Intramural Research

Member, Editorial Advisory Board, Environ Res.

Adjunct Professor of Biostatistics, University of Pittsburgh Graduate School of Public Health

Member, American Association for Cancer Research Committee on Environmental Carcinogenesis

Member, Working Cadre of National Large Bowel Cancer Project

Member, Epidemiology Committee, Breast Cancer Task Force

Levin, D.L.:

Member, Clinical Trials Contract Review Committee, Chemotherapy, NCI

Mantel, N.:

Member, International Statistical Institute

Visiting Professor, Department of Statistics, University of Tel Aviv, Tel Aviv, Israel

Lecturer and/or Consultant at various institutions in Israel including: Department of Social Medicine, Hadassah Medical Center; Department of Statistics, Hebrew University, Jerusalem; Volcani Agricultural Research Institute.

Adjunct Professor of Biostatistics, University of Pittsburgh Graduate School of Public Health

Editorial Advisory Board Member, Cancer Research

Member, Technical Advisory Committee, Federal Advisory Board to the Higher Education Panel, American Council on Education

Publications

Brown, C.:

Godwin, J.D. and Brown, C.: Letter: Magnesium and Sudden Unexpected Infant Death. Lancet, 1176, May 26, 1973.

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Haenszel, W.:

See Individual Project Report NCI-4258

Levin, D.:

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Mantel, N.:

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Kende, M., Goldberg, A.I., Glynn, J.P., Mantel, N. and Goldin, A.: Relationship between white blood cell count and diagnosis and therapeutic response in AKR mice with spontaneous leukemia (lymphoma). Cancer Chemotherapy Reports 56, 683-690, 1972.

Mantel, N. and Brown, C.: Letter: Combining of independent regression estimates is not paradoxical. The American Statistician 27, No. 3, 125-126, 1973.

Myers, M.H., Hankey, B.F., and Mantel, N.: A logistic-exponential model for use with response-time data involving regressor variables. Biometrics 29, 257-269, 1973.

Schneiderman, M.A. and Mantel, N.: The Delaney clause and a scheme for rewarding good experimentation. Preventive Medicine 2, 165-170, 1973.

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Mantel, N. and Brown, C.: A logistic reanalysis of Ashford and Sowden's data on respiratory symptoms in British coal miners. Biometrics 29, 649-665, 1973.

Mantel, N.: Approaches to a Health Research Occupancy Problem. Biometrics. In press.

Goldin, A., Carter, S., and Mantel, N.: Evaluation of antineoplastic activity: requirements of test systems and statistical design. Handbook of Experimental Pharmacology - Antineoplastic and Immunosuppressive Agents. In press.

Goldin, A., Venditti, J.M., and Mantel, N.: Combination chemotherapy: basic considerations. Handbook of Experimental Pharmacology - Antineoplastic and Immunosuppressive Agents. In press.

Mantel, N.: Therapeutic Synergism. Cancer Chemother. Rep. In press.

Levy, Paul S., and Mantel, N.: Combining unbiased estimates - a further examination of some old estimators. J. of Statistical Computation and Simulation. In press.

Mantel, N., and Byar, D.: Evaluation of response-time data involving transient states: An illustration using heart-transplant data. JASA, March 1974.

Mantel, N.: Discussion of Conover. JASA, June 1974.

Mantel, N., and Li, C.C.: Estimation and testing of a measure of non-random rating. Ann. Hum. Genet. In press.

Mantel, N., and Brown, C.: Alternative tests for comparing normal distribution parameters based on logistic regression. Biometrics, September 1974.

Murray, J.:

Murray, J., and Axtell, L.: Guest Editorial: Impact of Cancer: Years of Life Lost Due to Cancer Mortality. J. Nat. Cancer Inst., Vol. 52, No. 1, 3-7, January 1974.

Serial No. NCI-4254

1. Biometry Branch, OADFS&S
   Division of Cancer Cause and Prevention
2. Bethesda, Maryland

NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on Cancer in Defined Populations: Surveillance, Epidemiology, and End Results (SEER) Program

Previous Serial Number: Same

Principal Investigators: S.J.Cutler, H.Geller, M.H.Myers, J.L.Young,Jr.

Other Investigators: R.R.Connelly, B.F.Hankey, W.I.Lourie,Jr., J.L.Murray

Cooperating Units (SEER Program Participants):

California State Department of Public Health
Connecticut State Department of Health
Fred Hutchinson Cancer Research Center (Seattle)
Research Corporation of the University of Hawaii
University of Iowa
Louisiana Social and Rehabilitation Services Administration
Michigan Cancer Foundation
University of New Mexico
University of Utah

Man Years:

Total:	6
Professional:	3
Other:	3

Project Description

Objectives:

To provide descriptive data on cancer incidence and to investigate the variation in risks for a standard range of demographic variables including region, place of residence, race, nativity, economic status and occupation; to identify promising leads and observational settings for analytical studies to test specific hypotheses on etiological relationships.

Methods Employed:

The descriptive incidence data come from a group of population-based registries covering defined geographic areas which record for each cancer pertinent data on a standard list of characteristics including age, race, sex, place of

residence, cancer site and histologic type, and which periodically produce statistical reports and supply data on individual patients in accordance with a uniform code to the Biometry Branch. Some participants include epidemiologic research units which develop specific epidemiological hypotheses suggested by an analysis of morbidity and mortality data. They develop the study protocol and may pretest the instruments for data collection. From these preliminary investigations, proposals are generated which can be submitted for financial support either by government agencies through grants or contracts, or by nongovernmental institutions or agencies. The full investigation may be a cooperative activity involving a number of SEER programs.

Major Findings:

The SEER Program is a recent development, and most of the effort has been in initiating new population-based registries to give the program wide geographic coverage and to include areas that have large segments of minority populations such as Blacks, Spanish Americans, American Indians, and mixed ethnic groups, so that sufficient data can be obtained for meaningful analysis.

It is anticipated that all of the programs will be in full operation by the end of 1974. Under the proposed timetable of operations and data submission, it is anticipated that data will be available less than 18 months after the end of the reporting period (1975 incidence data will be available by the middle of 1976).

The first epidemiologic investigations under the SEER Program were developed by the California Resource for Epidemiologic Research. A study protocol has been developed for investigating the association between fertility and breast cancer. The study population of breast cancer patients will be identified from cases reported to the registry. Control cases will come from hospital patients with a diagnosis other than breast cancer, or matched neighbors. Another study under way is investigating the contribution of diet and other environmental factors to the rapid increase in cancer of the colon/rectum in the Black population. Here again the study population will be drawn from cases reported to the registry, while control cases will be hospital patients and matched neighbors. This study is being conducted by the Kaiser Research Foundation with cooperation by the California registry.

Significance of the Program to the Institute:

Continuing information on incidence from representative groups of the U.S. population is required so that the nature and magnitude of the cancer problem including changes over time can be determined. Through close scrutiny of variations in cancer incidence with respect to geographic and demographic characteristics of the population and changes over time, specific etiologic hypotheses will emerge for testing which may lead to the identification of controllable risk factors. Continued recording of cases will also provide for the evaluation of cancer control activities. Comparisons could be made for such factors as changes in stage of disease before and after the institution of the control program compared to those detected through usual medical practice, etc.

Proposed Course of the Project:

1. The SEER Program is dependent on such factors as complete reporting of all cases with active disease treated in the reporting area or resident of the reporting area. Attention will be directed to ascertain the degree to which this is accomplished, and to setting up procedures to achieve and maintain a very high level of complete case reporting. NCI has also established staff resources to assist the programs in developing a high degree of efficiency in record handling and data processing, as well as to insure that the data submitted to NCI meet standards of completeness, accuracy, and comparability. To assist in this phase of the staff operation, a contract will be negotiated with the University of California Medical School in San Francisco where personnel experienced in registry operations are available, and where there is a highly recognized program for the training of registry personnel.

2. The Connecticut and California registries will continue to provide data on cancer incidence and mortality. Connecticut has been collecting these types of data since 1935. California has information for Alameda County back to 1960, and for the other four counties since 1969 when the Third National Cancer Survey was started.

3. Detroit is now part of the reporting system and will attempt to do follow-up on all cases identified in the Third National Cancer Survey and are not known to have died.

4. The Iowa registry is a combination of the University Hospital, which was part of the End Results Program, and the rest of the state which was part of the Third National Cancer Survey. Procedures are being worked out, and population-based data will be obtained starting with cases diagnosed in 1974.

5. Utah and New Mexico registries are in the process of converting operations from one which was primarily oriented to service to the cancer patient, to one which can also be used to generate incidence data and have an epidemiologic research potential.

6. The registries in Seattle, Hawaii and New Orleans are still in the developmental stage, but should be fully operational before the end of the fiscal year. Data from these registries will be obtained for 1974, and the completeness and accuracy evaluated.

Serial No. NCI - 4257

1. Biometry Branch, CADFS&S, DCCP
2. End Results Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: STUDIES OF THERAPEUTIC END RESULTS AND PROGNOSTIC FACTORS

Previous Serial: Same

Principal Investigators: Sidney J. Cutler, Max H. Myers

Other Investigators: William I. Lourie Jr., Lillian M. Axtell, Herman Heise, Ardyce J. Asire, Evelyn M. Shambaugh, Benjamin F. Hankey, Anne Baranovsky, Philip Prorok, Charles C. Brown, Debra T. Silverman, David Godwin, Sylvan Green.

Cooperating Units: California Tumor Registry; Connecticut Tumor Registry; Massachusetts Cancer Registry; Charity Hospital of Louisiana; Indiana University Medical Center; State University of Iowa Hospital; University of Chicago Clinics; University of Michigan Hospital; University of Virginia Hospital.

Man Years:

Total:	15
Professional:	9
Other:	6

Project Description

Objectives:

To assist other Branches of the National Cancer Institute, other health agencies, and treatment centers in the development and maintenance of programs for the collection and analysis of data on cancer in human populations with special emphasis on the end results of treatment. To cooperate in international studies for the purpose of examining the relationship of etiologic and prognostic factors in cancer. This includes consultation and advice on methodological problems, development of improved statistical tools for the analysis of observations on human populations, and active collaboration in the collection and analysis of data in a continuing collaborative program and in specific projects.

Methods Employed:

1. The End Results Section staff provides advice on the development and application of uniform definitions and procedures to participating cancer registries, provides consultation on planning and implementing special studies, edits and analyzes data submitted to the National Cancer Institute in accordance with a Uniform Code, and explores more efficient data processing and statistical techniques to abstract the maximum amount of useful information from the collected data.

2. The program draws on standard statistical and actuarial techniques and accepted principles for records management, with necessary modifications required for any individual study situation.

Major Findings

1. An analysis of data on 18 forms of cancer for 219,493 white and 21,088 black patients diagnosed during the 10-year time period 1955-64 indicated that the level of survival for black patients was lower than that for white patients and for both races women had a higher level of survival than men. In general, the largest difference in the level of survival between whites and blacks was observed for the forms of cancer with the better prognosis such as cancers of the bladder, larynx and uterine corpus. For the more lethal forms, such as stomach and lung cancer, there were small differences in survival. The 5-year relative survival rates for black and white cancer patients, for all stages and for localized disease, are shown in the following table.

	5-Year Relative Survival Rate			
	All Stages		Localized	
	White	Black	White	Black
Corpus	72	40	83	63
Female breast	62	47	84	77
Cervix (invasive)	60	47	79	78
Bladder	56	28	69	47
Larynx	55	45	73	59
Prostate	51	41	64	58
Colon	46	36	73	66
Rectum	40	30	64	60
Hodgkin's disease	39	26	-	-
Kidney	36	40	62	69
Ovary	32	28	72	74*
Brain and cranial meninges	28	28	30	30
Chronic leukemia	27	19	-	-
Stomach	12	10	39	42
Multiple myeloma	9	9	-	-
Lung and bronchus	8	6	30	23
Esophagus	3	2	11	6
Pancreas	2	2	7	5
Acute leukemia	1	2	-	-

* Rate has a standard error between 5 and 10 percent.

For some forms of cancer, where the disease is localized to the organ of origin, such as bladder cancer, the difference in survival between black and white patients seemed to be limited to the first few years after diagnosis. For patients with other forms of cancer, prostatic cancer, for example, black patients continued to have excess mortality throughout at least 10 years. Factors which could play a role in explaining the poorer survival among black patients should be explored, e.g., overall quality of medical care, spectrum of histologic types, and immunological reactions to the cancerous process.

2) Hodgkin's disease diagnosed among 1,633 Connecticut residents during 1940-69 was studied systematically for the presence of 1- and 2-year clusters. To eliminate the effects of increases in incidence of the disease and changes in the composition of the population, 1940-69 was studied as 6 contiguous 5-year groups. The data indicated some clustering, though not highly significant, for 1950-54 and 1955-59. A more uniform distribution of cases than expected for 1965-69 was also indicated. No real evidence of clustering for the entire study period was apparent.

3) In order to evaluate the feasibility of international studies of Hodgkin's disease patients, the reliability of the diagnosis of histologic subtypes by 16 pathologists from several countries was tested. The Rye classification was applied to a set of 40 slides, 20 of which were read twice. The inter-observer agreement varied with the histologic subtype: for the nodular sclerosis subtype, agreement in diagnosis was 70% in the first reading and 91% in the second reading. For the lymphocyte predominant subtype, agreement varied between 68 and 94% in the different readings. For the mixed cellularity and lymphocyte depleated subtypes, agreement varied between 58 and 85%. Pathologists least agreed on diagnosis when slides previously thought difficult to classify were included. This was a cooperative study with the laboratory of Pathology, NCI.

4) A theoretical framework for the design and analysis of screening programs for the early detection of chronic disease is being developed. The research to date has concentrated primarily on the periodic screening of a large population to detect a progressive chronic disease. An idealized model for the natural history of such a disease is assumed wherein the disease proceeds from a preclinical to a clinical state. In probabilistic terms the model is a stationary stochastic point process. Distribution theory of bounded recurrence times of this point process has been worked out as a foundation for the methodology. The distribution and mean of the lead time resulting from, and formulas for the proportion of preclincial cases detected by, periodic screening have been derived. These expressions are functions of the time interval between screens, the number of screens, and other parameters important in the design of such screening programs. Furthermore, equations have been derived for use in the analysis of data from a periodic screening program. In particular, estimates of the mean lead time and the probability of detecting preclinical disease have been obtained. A key result is an estimate of the mean lead time for those preclinical cases detected at any particular screen in the sequence. Under the assumption of perfect detection, this is expressed completely in terms of quantities estimable from data: prevalence and incidence rates and ages at diagnosis.

5) A mathematical model utilizing the geometric distribution for analyzing time-to-response data (survival data) has been developed. This model takes into account concomitant information by means of a multivariate logistic function. A parameter introduced into the model to make the regression estimates independent of choice of time scale also serves as a model specification parameter. Allowing this parameter to take on its limiting values of zero and infinity results in two distinct exponential models involving regressor variables as described by other investigators.

The model has been used to analyze data on recurrent advanced breast cancer patients where the primary interest was in examining the relationship of sites of involvement and disease-free interval to survival subsequent to dissemination. Both factors were found to be prognostically important. The model is also being used to examine the relationship of a large number of urinary steroids to survival subsequent to adrenalectomy for patients with disseminated breast cancer. The model should be of use in identifying patient characteristics that imply poor patient survival after treatment by adrenalectomy.

Significance to the Program of the Institute:

Many of the benefits of the consultation program are long range in nature and consist of setting up record systems and study situations which enlarge the potential study resources of NCI and related organizations. The research objectives of the Institute are promoted by continued work on the integration of various study techniques, e.g., retrospective and prospective studies, analyses of mortality data and morbidity surveys, epidemiologic investigations and cancer registries. An example of the interaction between epidemiologic and end-results investigations are the developing studies of prognostic factors and the role of these factors in cancer etiology.

Proposed course of the project:

1. This project is part of the Collaborative Program for Cancer Surveillance, Epidemiology and End Results Reporting (SEER Program) being developed within the Biometry Branch. Emphasis will continue to be placed on obtaining and reporting data on cancer therapy and associated survival patterns. The base for reporting of end results is being expanded by the addition to the SEER program of registries covering the incidence of cancer in defined populations. New Orleans, Detroit, Utah and Seattle are the participants most recently added to the program.

2. Activities in the development of special studies on prognostic factors will continue. In some cases these studies will be in collaboration with the American Joint Committee for Cancer Staging and End Results Reporting, in others with cancer specialty centers such as Memorial Hospital (N.Y.C.). With the development of the Resource for Cancer Epidemiology associated with the California Tumor Registry and the Research Unit in Connecticut, the potential for an enlarged special study program will be greatly increased.

3. An expansion of project activities is expected in the area of international collaboration. A pilot study of extent of disease and survival distributions among countries with varying levels of incidence for cancers of the breast and colon is underway. This study should reveal the feasibility of conducting detailed studies to determine the relationship between factors associated with levels of incidence, the degree of cancer spread at diagnosis, and survival subsequent to diagnosis. Representatives from Finland, Norway and Birmingham, England have indicated an interest in this type of study and have agreed to participate in an assessment phase of a study of breast and colon cancer.

Publications

Correa, P., O'Conor, G.T., Bernard, C.W., Axtell, L.M. and Myers, M.H.: International Comparability and Reproducibility in Histologic Subclassification of Hodgkin's Disease. JNCI 50: 1429-1435, 1973.

Kryscio, R.J., Myers, M.H., Prusiner, S.T., Heise, H.W. and Christine, B.W. The Space-Time Distribution of Hodgkin's Disease in Connecticut, 1940-69. JNCI 50: 1107-1110, 1973.

Newell, G.R., Roberts, J.D. and Baranovsky, A., Retinoblastoma: Presentation and Survival in Negro Children Compared With Whites. JNCI 49: 989-992,1972.

Baylor, S.M. and Berg, J.W.: Cross-Classification and Survival Characteristics of 5,000 Cases of Cancer of the Pancreas. J. of Surg. Oncol: 335-357, 1973.

O'Conor, G.T., Correa, P., Christine, B., Axtell, L.M. and Myers, M.H.: Hodgkin's Disease in Connecticut: Histology and Age Distribution. International Symposium on Hodgkin's Disease. NCI Monograph 36: 3-8, May 1973.

Murray, J.L. and Axtell, L.M.: Impact of Cancer: Years of Life Lost Due to Cancer Mortality. JNCI 52, No. 1: 3-7, January 1974.

Serial No. NCI-4258
1. Biometry Branch, OADFS&S
   Division of Cancer Cause
   and Prevention
2. Demography Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of Cancer Incidence and Related Etiologic Factors

Previous Serial Number: Same

Principal Investigator: William Haenszel, Haitung King, and John Young

Other Investigators: Roger Connelly, Susan Devesa, Gary Glober, Frances Locke, Constance Percy, Joseph Scotto Roger Williams

Cooperating Units: University of California Medical School
New England Deaconess Hospital
Connecticut Tumor Registry
Aichi Cancer Institute, Nagoys, Japan
Kuakini Hospital, Honolulu
Cancer Registry of Norway
Universidad del Valle, Cali, Columbia
Framingham Field Unit, NHLI

Man Years:

Total:	12
Professional:	7
Other:	5

Project Description

Objectives:

To describe cancer morbidity and mortality in human populations; to seek out and examine significant differences between persons who develop cancer and those who do not; to search for clues to causative or promoting factors of cancer by comparisons between high- and low-risk populations; an example of the latter being certain migrant groups in the U.S. useful for the study of host and environmental effects in specific cancer sites; to relate epidemiologic features of cancer to survival rates; to identify personal and environmental factors of potential significance in the prevention, control, early diagnosis, or successful treatment of cancer.

Method Employed:

These studies require an extremely wide variety of methods of data collection and analysis, and in many cases new methods must be developed. Where appropriate, these are described in the following sections.

Major Findings:

The Migrant Studies Program currently consists of four components: Norwegian, Japanese, Colombian and Polish (sponsored by PL-480 funds in Poland). Observations have established the nature of the changes in cancer-risks in these migrant populations. In all populations efforts are directed toward obtaining comparative epidemiologic and supporting pathological data while exploiting the special capabilities of each collaborator and to take advantage of the experiments of nature represented by migration. The major efforts to date have been directed to stomach, large bowel and prostate.

Stomach. The relation between cancer and premalignant conditions of the stomach has received special attention in Colombia, Hawaii and Japan and the distinction between intestinal and diffuse types of stomach cancer has helped clarify the role of intestinal metaplasia of the gastric mucosa as a possible precursor lesion. Recent review of stomach cancers among Japanese in Hawaii and Miyagi prefecture, Japan revealed that age slopes for log incidence were different for intestinal and diffuse type cancers. For the intestinal type the native and migrant Japanese had the same age slopes, but the curve for Hawaiian-Japanese was displaced to the right. Such observations are helpful in development of hypothesis on pathogenesis.

A cohort of Japanese males in Hawaii assembled by the National Heart and Lung Institute is being studied for gastrointestinal cancer. One major objective of the cohort studies is an attempt to transform the epidemiology of stomach cancer into the epidemiology of a potential precursor -- intestinal metaplasia. Observations in Colombia, Japan and Poland all agree on strong associations in the distribution of gastritis, gastric ulcers, metaplasia and gastric cancer, much different from the prevailing U.S. picture, where there is no evidence for any association between gastric ulcers and cancers.

Colon. A case-control study in the Hawaiian Japanese--a population undergoing displacement from low to high colon cancer risk and in varying stages of transition from Japanese- to Western-style diet--has uncovered important correlations between food items and bowel cancer (JNCI 51:1765-1779). The major findings were:

a. More bowel cancer patients than controls had abandoned the practice of eating at least 1 Japanese-style meal daily.

b. Patients ate meat, legumes, and starches more frequently, with the disparity between patients and controls being less pronounced for starches than for meat and legumes. When the relative risks for individual meats and legumes were adjusted for consumption of other foods within the same category, beef and string beans were identified as major contributors to the meat and legume effects.

c. The case-control differences for meat (beef) and legumes (string beans) persisted among both Issei and Nisei; the excess frequency in consumption of starches was limited to Nisei.

d. Individual meats, legumes, and starches displayed gradients in risk rising with frequency of use.

e. Separate, independent effects for beef, string beans, and starches were suggested; the case-control contrasts were strengthened by the consideration of various combinations of beef, string beans, and starches.

f. The associations with beef and string beans were enhanced in the subset of patients whose tumors were in the sigmoid and adjacent colon.

Several points of consistency between this study and other evidence based on international comparisons of populations at high- and low-risk to this disease are evident. The findings on beef appear to have the most immediate relevance and the major significance of this study is that the findings may narrow the area of search for etiologic factors and assist in the planning of future research on the complex sequence of events leading to bowel cancer.

Additional studies of the distribution of intestinal polyps in high- and low-risk populations for large bowel cancer in New Orleans, Honolulu (Japanese) and Japan expanded on the findings for cancer of the colon and rectum and adenomatous polyps published by Haneszel and Correa. Stemmerman and Yatani (Cancer) have reported the distribution of both the adenomatous and hyperplastic polyps among Hawaiian Japanese to parallel closely the New Orleans experience, while unpublished data from Japan portray a low prevalence of both adenomatous and hyperplastic polyps, which parallel the previous results from Colombia. There can be no doubt that among Japanese migrants a profound change in the number and the anatomical distribution of adenomatous and hyperplastic polyps has accompanied the marked rise in colon cancer risks in the migrant Japanese.

Prostate. The work of Akazaki (JNCI 50:1137-1144, 1973) offers the possibility of resolving anomalous observations on the prevalence of occult prostatic carcinoma and clinically active disease for Japanese populations at home and abroad. Akazaki distinguished between two forms of occult disease -- proliferative,

non-proliferative -- and was able to demonstrate a rise in prevalence of the proliferative type among Hawaiian-Japanese consistent with their increased incidence of clinically active disease. An effect limited to a subset of more aggressive appearing occult lesions suggests a role for some unidentified promoting factor. These findings have led to the design of a case-control study in Hawaii and Japan, supplemented by collection of additional data on the cohort of Japanese males being followed in Hawaii, by investigators associated with the ongoing contract-supported program (Drs. Rhoads and Avki). A proposal has been submitted to the Prostate Cancer Task Force for consideration and possible funding.

The Framingham Cancer Study is a joint project of NCI and NHLI. From the cohort of 5,209 patients followed for 22 years in the Framingham Heart Study all patients with cancer are being identified. Pertinent information (date of cancer diagnosis, type and site from pathology reports, etc.) is obtained for each cancer patient. When complete this roster of cancer patients will be compared to the noncancer patients in the cohort with respect to many interesting variables followed during the 22 year heart study. Variables include historical items (alcohol and coffee consumption, smoking, education, birth control pills, number of offspring, etc.); physical findings (abnormal lymph nodes, obesity, eye color, etc.); laboratory findings (serum cholesterol, blood type, chest x-ray, etc.); and diagnosis (rheumatoid arthritis, diabetes, hypertension, etc.).

The overall purposes are to:

(a) test known hypotheses (e.g., cholesterol and colon cancer)

(b) find possible new associations to form new hypotheses and stimulate other research (e.g., drugs vs. cancer types)

(c) in selected cancer patients, analyze frozen sera saved from before their diagnosis to investigate the time course of tumor associated antigens.

Development of the National Cancer Program has reinforced the need for a U.S. network of cancer registries for information required for planning and administration and to supply continuing data on incidence and treatment end results. Much time has been spent in the past year on implementation of the recommendations of the Advisory Committee on Population-based Epidemiology Research Centers.

NCI has developed a coordinated program of cancer surveillance, descriptive epidemiology and end-results evaluation. The output of this multi-purpose program will be the basis for:

(a) prompt identification of secular trends in specific cancers;

(b) identification of variation in disease incidence that may lead to etiological hypotheses;

(c) assessment of the efficiency and quality of the medical care system;

(d) studies by which primary and secondary preventive measures can be accurately measured.

Localities participating in the Third National Cancer Survey were evaluated as potential candidates for the expanded registry network because they have professional and technical personnel trained in relevant methodology, and because of their established rapport with the medical community without which no registry can succeed. Three areas - Detroit, Iowa, San Francisco Bay Area - were selected as participants. Atlanta is also being considered as a participant. Additional participants have also been selected (see NCI-4254).

The descriptive incidence data from population-based registries will lay the groundwork for investigations focused on etiology, either in the form of analytic epidemiological studies or animal experiments.

The incidence and surveillance objectives enumerated require both:

(a) Population-based cancer registries. These will provide the bulk of the descriptive incidence and end results data.

(b) Population-based Cancer Study Centers. In addition to maintaining surveillance and reporting on incidence and end results, these centers will conduct epidemiological and clinically-oriented studies. They will augment the conventional registry effort with diagnostic, therapeutic, epidemiologic, statistical, laboratory, and other skills on which valid data on incidence and end results depend. Some Population-based Cancer Study Centers may be part of a Comprehensive Cancer Research Center; others may be closely allied with strong departments of medicine, surgery, and pathology with special interests in cancer. The Population-based Study Centers will enhance the opportunities to conduct controlled clinical trials among truly representative samples of cases.

NCI research and program goals do not presently require, or even suggest an ultimate need for, the establishment of a large scale program, national in scope, of cancer registration.

The coordination of work in cancer epidemiology to be carried out by population-based cancer study centers and comprehensive cancer research centers does present a completely new dimension for a national program. The existing model for data handling represented by the End Results Group is not relevant, since each center will

be engaged in a broad panorama of multidisciplinary research and training activities not amenable to centralized direction and control. The mechanism to facilitate exchange of information among the research centers and to enable them to develop programs compatible with broad national goals will be a committee which will include the directors of the several research centers as members. The first formal meeting of this committee is being held in Berkeley, California, March 20-21, 1974.

The study on Cancer Mortality Among Foreign- and Native-Born Chinese in the United States was completed. Apart from their well-known liability to nasopharyngeal carcinoma, the noteworthy characteristics of the Chinese from the vantage point of internal U.S. comparisons have been the low risk for prostate and high risks for liver and esophagus among males, and the low risk for breast and high risk for lung among females. The differences cited follow the general pattern described for Asian Chinese. Migration to the United States may have diluted the risks expressed for these sites by Asian Chinese, but to date this has not resulted in any fundamental change.

The rise of colon cancer mortality to the U.S. white level, conforms to the experience noted for migrants from Japan and from those European countries where low colon cancer risks have prevailed. The upward displacements noted for ovary and suspected on the basis of uncertain evidence for corpus uteri among Chinese females in this country also agree with the pattern previously observed for other migrants to the U.S. including those from Japan. We have noted that comparative population studies have revealed close associations in risks for breast, corpus uteri, and ovary, so that the failure of breast to rise in concert with the risks for corpus and ovary among U.S. Chinese appears striking and its significance seems enhanced by the fact that it corresponds precisely to the Japanese migrant experience.

Although the inability to demonstrate clearcut idai-erdai differentials for many cancer sites may be a function of the small numbers of death observed, this may not be the complete answer. We have noted that nativity may be confounded with place of origin in continental China and that for nasopharynx genetic differences among populations within China have not been ruled out as possible explanations. Information available from Chinese sources, while not definitive, suggest substantial regional variation in cancer risks by sites within that country (37). The geographic variation in site-specific cancer risks needs more thorough documentation and hopefully, more data for continental China will become available in future years.

The findings in hand suggest that the Chinese represent a resource for the study of cancer epidemiology that may be turned to advantage. They have migrated to many countries throughout the world and the diversity in new environmental backgrounds may yield clues on the interplay of environmental factors and host

characteristics in carcinogenesis, if the desired information could be assembled and collated.

Significance to the Program of the Institute:

This program helps establish baselines for cancer mortality and morbidity in various populations from which changes can be measured, ascertains trends in risks which may reflect the action of various etiologic agents, identified high- and low-risk population groups which can be studied and compared, and provides observations on human populations useful for correlation with laboratory studies. Ad hoc investigations are undertaken because of unusual or unexpected findings from the routine monitoring of incidence and mortality rates.

The migrant population studies represent a major biometric effort of the Institute. If these studies elucidate the environmental factors involved in the incidence of certain forms of cancer and indicate those which are predominantly environmental and those which are predominatly genetic, it may be possible to apply these findings to the United States population for purposes of cancer prevention and cancer control.

Proposed Course of the Project:

The migrant studies will emphasize analysis of data bearing on the pathogenesis of the major sites under study, particularly large bowel. The findings from the case-control studies of gastrointestinal cancers and diet will be used in developing the protocols for the ongoing cohort study of Hawaii Japanese males and the extension to cohorts in Japan. Field studies using the gastrocamera and other screening techniques of the population at very high risk for stomach cancer in one department of Colombia are receiving high priority. Greater efforts in the synthesis of data in hand and being collected are required and this will require closer collaboration with the principal investigators for the several related programs being supported by research contracts.

Incidence data for the year 1973 will be submitted to NCI by SEER program participants in January, 1975. These data will be analyzed by staff to investigate both geographic differences and time trends in incidence by site and type. Comparison with data from the Third National Cancer Survey will be possible and in addition to serving the purpose of formulating time trends, such comparisons will serve as quality control checks for programs in the SEER program but not in the survey.

The immediate course of the other projects is not predictable, since many are undertaken as the need becomes apparent and as staff time is available. A number of studies are in various phases of completion, and the items listed below represent long-term efforts and shortage of staff resources will delay completion until at least the following year.

A study is being planned on consumption of herbs among the Chinese in several major centers of Chinese settlement including San Francisco, Singapore, Taiwan, and Hongkong. Field studies are being contemplated in Continental China in regard to selected cancer sites with high risk among the Chinese. Thoughts are also being given as to the possibility of collecting massive cancer data from various parts of New China.

Data have been collected on non-cancer deaths among the Chinese in the same areas described under Major Findings, and will be useful for checking the validity of findings on the relationship between stomach malignancy and duodenal ulcer in Chinese populations. Plans are being made for updating information on various aspects of cancer in Mainland China and for investigating consumption of herbs among the Chinese in selected areas.

Investigation of mortality among defined cohorts of clergymen will be continued. Analysis to date demonstrates a reduced risk of death in all age groups and for most major causes of death, including many cancer sites. While the reasons for these reduced risks are not yet known, further accumulation of massive data at both national and international levels would provide useful clues for testing specific hypotheses.

Ten years of follow-up data are available for 3,500 polyp-free patients, and fifteen years of follow-up data have been collected on an additional 500 patients, all examined in the mid-1950's at Lahey Clinic, Boston. The Lahey Clinic has collected fifteen years of follow-up data on 300 patients examined during the same time period found to have rectal or colonic polyps at examination. Both the polyp-bearing and polyp-free groups experienced fewer than expected colon and rectal cancers during the first ten years post examination. However, the polyp-bearing group showed a far greater than expected number of these cancers during years eleven to fifteen post examination, so that it is of extreme importance to complete the fifteen-year follow-up for the entire polyp-free series to permit accurate comparisons between the two groups. This will be undertaken as staff is available.

Honors and Awards:

King, Haitung:

Advisor - Consultant, D.C. Interagency Council on Smoking

Clinical Professor of Community Medicine and International Health, Professorial Lecturer and Research Associate in Demography, Georgetown University

Reviewer for Cancer

Organizer for a symposium on "What we do and don't known about China's public health" at the annual meeting of the American Public

Honors and Awards: (continued)

King, Haitung: (continued)

Health Association, San Francisco, November 4-8, 1973.

Invited participant at the Conference on China's Medical and Public Health sponsored by the National Academy of Sciences and the Johnson Foundation, Racine, Wisconsin, November 28-30, 1973.

Invited paper on "Epidemiology of Selected Diseases Among the Chinese" presented at the Conference on the Comparative Study of Traditional and Modern Medicine in Chinese Society, sponsored by the Fogarty Center and the University of Washington, Seattle, February 4-6, 1974.

Scotto, Joseph:

Founding member, International Lung Cancer Group

Young, John L., Jr.:

Contract Review Committee, Cancer Control Program, NCI

Publications:

Connelly, Roger R. and Christine, Barbara W. "A Cohort Study of Cancer Following Infectious Mononucleosis" Cancer Research - in press.

Haenszel, William "International Collaborative Studies of Cancer in Man: The Role of the National Cancer Institute" National Cancer Institute Monograph 40: in press.

Haenszel, William, Berg, John W., Segi, Mitsuo, Kurihara, Minoru, and Locke, Frances B. "Large-Bowel Cancer in Hawaiian Japanese" Journal of the National Cancer Institute 51:1765-1779, 1973.

Haenszel, William and Correa, Pelayo "Cancer of the Large Intestine: Epidemiologic Findings" Diseases of the Colon and Rectum 16:371-377, 1973.

Haenszel, William and King, Haitung "Cancer Mortality Among Foreign- and Native-Born Chinese in the United States" Journal of Chronic Diseases 26:623-646, 1973.

Percy, Constance L., Garfinkle, Larry, Krueger, Dean and Dolman, Alice "Apparent Changes in Cancer Mortality in 1968 as a Result of the Introduction of the 8th Revision, International Classification of Diseases" Submitted to Public Health Reports.

Young, John L., Jr. "Prevalence of Cardiorespiratory Symptoms Among Norwegian Migrants to the United States and Siblings Still Alive

Publications: (continued)

in Norway with an Adjustment for Sibship Size" Doctoral dissertation, University of North Carolina, April, 1974.

Serial No. NCI 4260
1. Biometry Branch, OADFS&S
   Division of Cancer Cause and Prevention
2. Clinical and Diagnostic Trials Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Consultation on Clinical Trials and Diagnostic Methods

Previous Serial Number: Same

Principal Investigator: David P. Byar

Other Investigators: Mitchell Gail, Larry Muenz, Nell Sedransk

Cooperating Units: V. A. Cooperative Urological Research Group (26 hospitals)

Man Years:
Total: 7
Professional: 4
Other: 3

Project Description

Objectives

To provide consultative services in statistical and epidemiological methodology necessary for the various programs sponsored by the Veterans Administration Cooperative Urological Research Group. This includes assistance in the design, interpretation and evaluation of the clinical trials conducted by the Veterans Administration Cooperative Urological Research Group, and provision for studies operating concurrently with the clinical trials such as epidemiological studies.

Methods Employed:

Standard methods of biometry, statistics, probability, and epidemiology with necessary modification as required by the particular problem. New techniques are developed by the personnel working with the Section to handle specific problems.

Major Findings:

This Section assists the investigators in developing and evaluating methods of treatment for cancers of the male genitourinary tract. Such assistance

is generally acknowledged in the publication of findings of the medical investigators. New statistical techniques are published independently.

The Section is more deeply involved in six separate clinical trials:

(a) Main Prostate I, which compares the effect of two treatments (prostatectomy alone, and prostatectomy with estrogen) for Stages I-II, and of four treatments (estrogen, orchiectomy, estrogen plus orchiectomy, and placebo control) for Stages III-IV. The intake of new patients into this study was terminated on March 31, 1967. A total of 2,315 patients had been entered by that date.

(b) Main Prostate II, which also compares the effect of two treatments (prostatectomy alone and placebo control) for Stages I-II and of four treatments (three levels of estrogen and placebo control) for Stages III-IV. This study is designed as a sequel to Main Prostate I. Intake of new patients was terminated in June 1969 after 561 patients had been entered in the study.

(c) Main Prostate III, which compares placebo versus prostatectomy for operable Stage I-II patients. Inoperable Stage I-II patients are randomly assigned to placebo or 1.0 mg Diethylstilbestrol. All eligible Stage III-IV patients are randomized to one of four treatments: 1.0 mg. Diethylstilbestrol, 30 mg. Provera, 2.5 mg. Premarin, or 30 mg. Provera plus 2.5 mg. Premarin. As of September 1, 1973, 1042 patients had been entered in this study.

(d) Focal Carcinoma, which compares several treatments for very early prostatic lesions of the type commonly found in TUR or autopsy specimens from old men. There are now 175 patients in this study.

(e) Urinary Bladder I, which compares surgery alone, radiation alone, and combined therapy for bladder cancer. There have been 72 patients admitted.

(f) Urinary Bladder II, which compares transurethral surgery followed by oral placebo, oral pyridoxine, or topical installation of thio-TEPA in treating Stage I tumors of the bladder. Intake of new patients into this study was begun in November 1971. As of September 1, 1973, 69 patients had been entered in this study.

The data from two trials -- Main Prostate III and Urinary Bladder II -- are not yet sufficient for detailed study.

The results of Main Prostate I have already caused widespread changes in the general treatment of prostatic cancer, because of the finding that the standard estrogen treatment for prostatic cancer carries a very great risk of death from cardiovascular diseases. In addition, the clinical benefits with respect to the cancer are smaller than was generally believed. Overall mortality rates in Stages I, II, and III, and in "early" Stage IV are

substantially higher in estrogen-treated patients than in those not receiving estrogens. This hazard was not recognized before the results of this study were available.

During the past year, efforts in this project have been concentrated on three objectives: elucidation of the mechanisms by which estrogen exerts its harmful effects, classification of patients with respect to the probable benefits or losses associated with each treatment tested, and studies (Prostate II) on the effects of various doses of diethylstilbestrol.

Preliminary results from Main Prostate II indicate (1) that there is no evidence thus far to show that 5.0 mg. diethylstilbestrol is superior to 1.0 mg. in controlling the cancer in either Stage III or IV, (2) that in Stage III there are substantially fewer heart and cardiovascular deaths in 1.0 mg. diethylstilbestrol compared to the 5.0 mg. dose, (3) that in Stage IV both the 1.0 mg. and 5.0 mg. doses are more effective than the 0.2 mg. dose in controlling the cancer, and (4) that in Stage III the superiority of placebo over the 5.0 mg. dose (and to a lesser extent over the other two doses of diethylstilbestrol) is accounted for by the excess deaths from non-cancer causes in the estrogen treated patients. Taking all these rather complex relationships into account we see no justification for the continued use of 5.0 mg. diethylstilbestrol in either Stage III or IV.

At the time that Main Prostate I was organized there was no reason to suspect an adverse effect of estrogen on the cardiovascular system, so much important information was not collected and submitted in a uniform way. We are now attempting to gather some items retrospectively (pre-treatment EKG, history of heart disease, admission blood pressure, etc.) but the analysis will be difficult. Main Prostate II includes very careful study of the cardiovascular system before treatment and at each follow-up, and may eventually provide answers to this problem.

It must be noted that cardiologists have not accepted the estrogen findings as readily as urologists. Results of the VA study have been of concern to the National Heart and Lung Institute because of its involvement in the Coronary Drug Project in which several drugs (including estrogen) were actually being used in the hope of reducing mortality among men with a recent myocardial infarct. NHLI assigned a staff member to make a detailed review of our original records and other data on Stages I-II, where the adverse effect of estrogen is most marked. His review completely supported all of our published conclusions. In November 1970 the first report of the Coronary Drug Project appeared (JAMA 214: 1303, 1970). In this report, data were presented which indicated that 5.0 mg. conjugated estrogen daily led to a significant increase in non-fatal events of myocardial infarction, pulmonary emboli, and thrombophlebitis compared to the placebo group. This treatment has since been dropped from their study.

During the last year we completed and published an analysis of the Focal Carcinoma study and found that almost none of the patients had died of cancer of the prostate. Adjustment procedures revealed that survival of older patients who did not have radical prostatectomy was as good as that of the comparable group of younger patients from Study I who did, when age was taken into account.

An analysis of the Urinary Bladder I Study revealed no difference in the three treatment groups being compared. However, there were many flaws in the study, and the number of patients entered was small. We reported the negative findings, and pointed out some of the difficulties of conducting a successful clinical trial in a rapidly progressing disease such as this.

We have also used the data collected from Prostate Study I to examine the general methodological question of analysis of data from clinical trials when not all patients remain on the assigned treatments and how to compare the secondary treatments when the second phase treatment was not part of the original design. A detailed analysis of the data from Study I was completed and published in which we addressed ourself to these problems. Another study completed and published last year was an investigation of the effects of treatments in Prostate Study II on serum testosterone levels in patients with cancer of the prostate. Random samples of 30 patients from each of the stage treatment groups in stages III and IV were selected from the computer records and serum samples of these patients were sent to a special laboratory for testosterone determinations. Statistical analysis of the data carried on in the Section revealed that 1.0 mg. daily of diethylstilbestrol produced a drop in serum testosterone almost equivalent to that of the 5.0 mg. dose although the drop was not quite so prompt. This work is continuing and being extended to the treatments in Prostate Study III.

In addition to these six clinical trials the Section provides statistical support for ancillary studies performed on these same patients. Included among these are studies of various biochemical values and hormones measured in sera at various points in time with attention paid to the response of these variables to treatment and their usefulness in predicting survival. Another study involves measuring zinc concentrations in sera and in needle biopsies, correlating the latter with the histological appearance of the biopsy. Extensive attention has been devoted to collaboration with Dr. Donald Gleason in working out and testing a grading system for prostatic cancer. In the last year a new project has been undertaken to investigate the possibility of quantitating the extent of osseous metastasis so that a summary index may be used in predicting survival and measuring response to treatment. Finally, a detailed study of metastases found at autopsy has been completed in which the findings were correlated with histological type of the tumor and the effects of treatment.

Significance to the Program of the Institute:

This Section serves the members of the Veterans Administration Cooperative Urological Research Group by assisting in preparing and revising protocols, forms, and records; giving statistical guidance in the design and execution of clinical trials; processing and analyzing data that is received daily on patients in the various studies; and assisting the members of the Group with reports on the progress of the various studies.

In return for these services, the Clinical and Diagnostic Trials Section has access to the largest complete set of data available anywhere on the course of prostatic cancer. Over 2,300 patients have been entered in Main Prostate I with follow-up as long as ten years. These data are proving extremely useful and interesting not only in the comparison of treatment effects, but also in defining the general behavior of prostatic cancer in relation to a host of personal, clinical, and laboratory characteristics.

Proposed Course of the Project:

The cooperative studies undertaken jointly by the Veterans Administration and this Section are planned to be a self-generating sequential series to continue as long as fifteen years. Consultative services of this Section have expanded considerably over the past years as the data from Main Prostate I and II have ripened and as patients have accumulated in Main Prostate III.

In the coming year, we will continue to give most of our attention to the analysis of Main Prostate I and II, and especially of the patients in Stages III-IV, since the other stages do not yet include large numbers of patients with long-term follow-up.

Studies of specific cardiovascular effects of estrogen on patients in Main Prostate I have been undertaken, based on data obtained retrospectively from the four largest hospitals in the study. A great deal of effort has been put into preparing and editing these data and they are now ready for a detailed analysis using sophisticated biometric methods. Using data from Prostate Study II we examined the effect of the four doses of estrogen on blood pressure. Our analysis revealed a dose related change after six months of treatment in the patients' pulse pressures. This analysis is now complete and the results are being prepared for publication.

Because the amount of useful data collected in these studies is more than our present staff can adequately analyze, contracts for assistance in the analysis were offered to the Yale University Department of Epidemiology and Public Health. During the past three and a half years extensive work was done extending work originally begun by us on the use of a multivariate exponential survival model. Specifically, we have studied a step-wise procedure for eliminating variables from the analysis in order of their importance in predicting survival based on the likelihood function. A goodness-of-fit

test has been devised for checking the agreement of the model with the observed data, and the model has been tested by means of half-sample pseudoreplication. These results have been submitted for publication. We have also investigated the effects of including first order interactions in the model, and have found that with these data they do not add appreciably to the information. We have compared estimates of the variance of the parameters of the model based on the asymptotic variances with those obtained in half-sample pseudoreplication and found that when estimating three such variances on samples of only 100 observations, the two estimates were in good agreement.

An additional aspect of this work has been the investigation of the concepts of optimal treatment based on a patients presenting signs and symptoms. The application of the exponential model suggests that no one treatment is best for all patients at a given stage but rather that the treatment is best determined by taking into account additional covariate information. During the past year considerable attention has been paid to the theoretical aspects of this problem. As expected the work on this contract was completed in this its third year, ending in July 1973.

Although all members of the Section are involved to some extent in the activities described above, these projects have been the main responsibility of the Section Head. In addition members of the Section are involved in other projects concerned with investigating various aspects of general methodological interest, especially as appropriate to analyzing data from clinical and diagnostic trials. Dr. Larry Muenz, a Ph.D. in statistics, has been studying the properties of the Mantel-Haenszel chi-square statistic when used pseudo-sequentially in comparing survival distributions. He has also completed a study of alternative estimators for testing independence in 2x2 tables. Dr. Mitchell Gail, an M.D. with considerable sophistication in statistical methodology, has studied sample size determination problems appropriate for various clinical trial designs, developed a method for measuring the population benefits to be gained by reducing exposure to environmental carcinogens, and devised methods for comparing clinical trials which eventuate in several responses (for example, improvement or not for each of several signs or symptoms) for each patient. Dr. Nell Sedransk, a Ph.D. in statistics, has studied the allocation of sequentially available units in clinical trials and optimal stratification schemes for prospective randomized trials. She has also considered the discrimination or classification problem in face of unequal a priori probabilities and/or unequal loss functions.

Members of the Section have been involved in extensive activities as site visitors and consultants to various programs outside the branch. The Section Head is a member of the Breast Cancer Task Force Treatment Subcommittee and Chairman of the Etiology and Prevention Subcommittee of the National Prostatic Cancer Project Working Cadre. Dr. Muenz is a statistical consultant to the Basic Biology Committee of the Breast Cancer Task Force, and has consulted with outside agencies on the problem of asbestos as an environmental

carcinogen. Dr. Gail has acted as a statistical consultant to the NCI Committee on Immunotherapy and the NCI Committee on Diagnostic Radiology. He is a member and statistician for the NCI Committee on Immunodiagnosis. Dr. Sedransk has served on numerous occasions as a site visitor for various programs of the NCI, assisted in reviewing protocols for clinical trials, and designed forms for data collection. Last July she was appointed to the Cancer Clinical Investigations Review Committee which acts as a study section for cooperative investigations.

During the last year we have continued our contract with the Wolf Research and Development Corporation who assist us in analysis of the large amounts of data at our disposal by providing programming assistance. We are currently using a full time systems analyst and two full time programmers. This contract has been extended for another year. This relationship has been surprisingly convenient and productive.

Honors

David P. Byar, M.D.

Invited Address, XVI Congress of the International Urology Society, Amsterdam, July 1-6, 1973.

Panel Discussion, American Urological Association Meeting, St. Louis, May 19-23, 1974.

Mitchell Gail, M.D.

Invited Lecturer, Sixth Clinical Cooperative Group Conference, Roswell Park, Buffalo, New York, September 1973. "Methods in Competing Risk Analysis"

Invited Lecturer, State University of New York at Buffalo, Department of Statistics, November 1973. "A New Formalism for Competing Risk Analysis"

Invited Lecturer, New York University Medical Center, New York, New York, March 1974. "Applications of Competing Risk Analysis in Setting Limits on Exposure to Environmental Carcinogens"

Nell Sedransk, Ph.D.

Invited Lecturer, State University of New York at Buffalo, Department of Statistics, November 1973.

Invited Lecturer, McGill University, Department of Statistics, Montreal, Canada, December 1973.

Publications

Blackard, C. E., Byar, D. P., Jordan, W. P., and the Veterans Administration Cooperative Urological Research Group: Orchiectomy for advanced prostatic carcinoma: A re-evaluation. Urology 1: 553-560, 1973.

Byar, D. P. and the Veterans Administration Cooperative Urological Research Group: Survival of patients with incidentally found microscopic cancer of the prostate: Results of a clinical trial of conservative treatment. J. Urol. 108: 908-913, 1972.

Byar, D. P., Mantel, N., and Hankey, B. F.: Predicting survival in terminal cancer. British Journal of Medicine 189: 611, 1973.

Byar, D. P.: The Veterans Administration Cooperative Urological Research Group's studies of cancer of the prostate. Cancer 32: 1126-1130, 1973.

Byar, D. P., Huse, R., Bailar, J. C., III, and the Veterans Administration Cooperative Urological Research Group: A multivariate exponential model for predicting survival of prostatic cancer patients with time-censored data. JNCI 52: 321-326, 1974.

Byar, D. P.: Zinc in male sex accessory organs: Distribution and hormonal response. In Structure and Function of the Male Sex Accessory Organs. (Brandes, D., Ed.) Academic Press, Inc. (In press).

Gail, M. H.: Time-lapse studies on the motility of fibroblasts in tissues culture. In Ciba Symposium on Tissue Cell Motility, American Elsevier, N. Y., 1972.

Gail, M. H. and Gart, J. J.: The determination of sample sizes for use with the exact conditional test in 2x2 comparative trials. Biometrics 29: 441-448, 1973.

Gail, M. H., Boone, C. W., and Thompson, C. S.: A calcium requirement for fibroblast motility and proliferation. Exptl. Cell Res. 79: 386-390, 1973.

Gail, M.: The determination of sample sizes for trials involving several independent 2x2 tables. J. Chron. Dis. 26: 669-673, 1973.

Gail, M.: Power computations for designing comparative Poisson trials. Biometrics (In press).

Gail, M.: Value systems for comparing two multinomial trials. Biometrika (In press).

Hurst, K. S., Byar, D. P., and the Veterans Administration Cooperative Urological Research Group: An analysis of the effects of changes from the assigned treatment. J. Chron. Dis. 26: 311-324, 1973.

Kent, J. R., Bischoff, A. J., Arduino, L. J., Mellinger, G. T., Byar, D. P., and Hill, M.: Estrogen dosage of testosterone levels in patients with prostatic carcinoma. J. Urol. 109: 858-860, 1973.

Mantel, N. and Byar, D. P.: Evaluation of response-time data involving transient states: An illustration using heart-transplant data. (Accepted by JASA).

Newball, H. H. and Byar, D. P.: Does reserpine increase prolactin and exacerbate cancer of the prostate? A case control study. Urology 2: 525-529, 1973.

Serial No. NCI-4265
1. Biometry Branch, OADFS&S
   Division of Cancer Cause and Prevention
2. Mathematical Statistics and Applied Mathematics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Consulting in Statistics and Applied Mathematics

Previous Serial Number: Same

Principal Investigators: John J. Gart, Hugh M. Pettigrew, Robert J. Connor, Donald G. Thomas, M.W.J. Layard

Other Investigators: Jun-mo Nam, Alroy M. Smith

Cooperating Units: None

Man Years:
Total: 4.0
Professional: 3.5
Other: 0.5

Project Description

To provide consulting assistance and collaboration to NCI scientists on the applications of mathematical statistics and applied mathematics to the interpretation and analysis of scientific experiments.

Objectives:

The principal objectives are (1) to collaborate with NCI scientists on mathematical problems related to cancer research; (2) to provide consulting assistance in statistics and applied mathematics to NCI investigators; and (3) to accelerate the use of quantitative methodology in various aspects of the NCI intramural program and extramural program.

Methods Employed

The methodology of applied mathematics, mathematical statistics and probability are applied to biomedical problems. Often variations of existing techniques are developed to suit the special requirements of a particular problem.

Major Findings:

During this year the staff advised and collaborated with many investigators in the major divisions of research in the National Cancer Institute as well as some outside contractors and other investigators. The various projects are grouped below in terms of the Divisions and Areas of the projects.

Division of Cancer Cause and Prevention (DCCP) Tobacco Working Group

Several members of the staff have been actively involved in the analysis of research directed toward producing a less hazardous cigarette. A single experiment consists of 7,000 mice painted with tobacco smoke condensate for up to eighteen months. The statistical analysis is both extensive and complex. During the past year Dr. Gart has completed the adjusted correlation analyses of the chemical characteristics with the biological activity of the condensate for the first such experiment.

Dr. Layard has begun the analysis of the second study. Mr. Thomas has taken over the major data processing responsibility for this experiment. He has improved and revised the programs developed by DCRT for this purpose, as well as using once again the programs he wrote for the first such study. Mrs. Smith has written stepdown multiple regression programs that can be used in both these studies.

DCCP: Carcinogenesis

Mr. Nam and Dr. Gart have been analyzing the results of a feeding study in rats of seventeen environmental chemicals. This study was done under contract at Litton Bionetics by Dr. Borge Ulland. This section has been collaborating with Dr. Norbert Page and Dr. E.K. Weisburger. Thus far, particular attention has been given to the statistical analyses of Mirex and Saccharin. In this connection Dr. Gart has also analyzed previous mouse data on Mirex in conjunction with possible action on this pesticide to be taken by the Environmental Protection Agency.

Dr. Pettigrew has been assisting Dr. David G. Kaufman of the Carcinogenesis Area and H. Robert Baird of the Division of Computer Research and Technology in the development of an interactive computer system to retrieve and analyze data stored in the Carcinogenesis Bioassay Data System. Mr. Thomas has also provided computer programming advice on

this project. This system will be used by employees of Wolf Research, working under contract, to generate routine data summaries and preliminary analyses for Dr. Norbert Page' s area. Dr. Pettigrew and other members of our section will continue to provide expert statistical consulting as it is required in the future.

Dr. Connor continued to serve as statistician for the University of Utah group studying the effect of chronic suppression on physical and chemical carcinogens. This work is being done on a contract from the Biology and Immunology Segment, Carcinogenesis Program, NCI. He is doing the analysis of the experimental data and assisting in the design of the experiments. This work is expected to continue for several more years.

Dr. Pettigrew has also been working with Dr. Kaufman and Mr. Baird on the analysis of data from an experimental attempt to induce respiratory tumors in hamsters by cigarette smoke in conjunction with asbestos, NiO, CoO, and DEN. A final report on this experiment is being prepared.

Dr. Pettigrew is analyzing data from an experiment designed by Dr. Kaufman and Dr. Peter Swann of the Middlesex Hospital Medical School to study the production of Kidney tumors by DMN and MMS.

Dr. Pettigrew, in conjunction with Dr. Jane Taylor of the Endocrine Evaluation Branch and Dr. Norbert Page, Chief of the Carcinogen Bioassay and Program Resources Branch, has continued to provide statistical consultation on the analysis of data gathered under contract by Dr. Albert Segeloff of the Ochsner Clinic, New Orleans, La., to investigate the possible synergism between radiation and estrogen administration in the induction of mammary tumors in A x C rats. Two site visits have already been made, and one more is being planned in order to begin the final report of the results obtained so far.

Dr. Connor collaborated on a project on the intranuclear distribution of DNA repair in human diploid fibroblasts. This work was done with Dr. Harris of the Lung Cancer Branch and Dr. Michael Lieberman and Mr. Frank Jackson of the Experimental Pathology Branch. A manuscript has been submitted for publication.

Dr. Pettigrew continued to collaborate with Dr. Gerald Clamon of the Lung Cancer Branch in the design and analysis of polyacrilamide gel electrophoresis studies, and discussed a variety of other problems with him.

Dr. Pettigrew has been collaborating with Dr. C. Wesley Dingman of the Chemistry Branch to develop stochastic models for DNA synthesis and breakage in an attempt to fit existing experimental data. Further experiments will be devised to test the models as they are developed.

Dr. Pettigrew has consulted with two members of the Cytogenetics and Cytology Section, Drs. Nicolae Popescu and Brian Myhr, on topics relating to cell transformation by chemicals in vitro.

Mr. Nam has consulted with Dr. R.S. Yamamoto of Experimental Pathology Branch on the design of experiment to induce the colonic and stomach cancers in animals.

Dr. Connor continued to advise and assist Biology Branch scientists in the design and analyses of their experiments. The scientists include: Dr. Robert Bast, Dr. Edward Leonard, Dr. Monte Meltzer, Dr. Howard Smith, and Dr. Berton Zbar.

Dr. Connor and Dr. Gart reviewed manuscripts for the Bioassay Operations Segment. Dr. Gart was an invited participant of the Bioassay Operations Segment Advisory Group of the Second Annual Carcinogenesis Collaborative Conference in San Antonio in November 1973.

DCCP: Viral Oncology

Dr. Pettigrew assisted Dr. Gary Pearson of the Viral Biology Branch in the preparation of a manuscript reporting the results of an experiment demonstrating enhanced cytotoxicity of normal spleen cells against MSB and MSC cells in the presence of an immune serum with high antibody titer. The paper was published in Cancer Research.

Dr. Layard analyzed electron-microscopic autoradiography data of Dr. Gerald Suskind, Viral Biology Branch, dealing with the distribution of DNA label in chicken fibroblasts in early stages of infection with Rous sarcoma virus.

Dr. Pettigrew has consulted with Dr. Kelman Perk, a visiting scientist in the Viral Oncology area, on the design and data analysis of a proposed study of pulmonary carcinoma in sheep in Israel. This disease is the only known lung neoplasm which is thought to be transmitted in nature, and thus provides a unique opportunity for the application of epidemic models to real cancer data.

Mr. Thomas consulted with Dr. M. Paranjpe of the cell biology section of the viral biology branch on a problem comparing the immuno-suppressive reaction of various treatments on normal mice and immune mice.

Dr. Pettigrew has been working with Dr. Perk and Dr. Michael Chirigos of the Virus and Disease Modification Section on the analysis of data obtained from spleens of mice treated with LSTRA and various drugs. It

is anticipated that this will lead to a new method of quantitating the effects of chemotherapy in the LSTRA system.

Office of Cancer Control

Dr. Connor joined the Cancer Control Breast Cancer Demonstration Program as the statistical advisor for their cooperative study. The study involves over 25 hospitals in the U.S. with each screening and then following-up 10,000 women.

Division of Cancer Biology and Diagnosis

For Dr. Mearl F. Stanton, Laboratory of Pathology, Dr. Layard is investigating suitable methods of analysis of data from an experiment involving the induction of epidermoid carcinoma in the lungs of rats by cigarette smoke condensate, and from a similar experiment using pellets containing 3-methylcholanthrene. Dr. Layard is also assisting Dr. Stanton in the analysis of a study of the relation between asbestos and fibrous glass particle size and mesothelioma induction in rat lungs.

Dr. Connor assisted Devron Char, Tumor and Cellular Immunology Section, LCBGY, NCI in planning a study to determine the efficacy of BCG in patients with malignant melanoma or in patients who have been enucleated for ocular malignant melanoma.

Dr. Pettigrew has completed the analysis of the data obtained by Dr. Pietro Gullino, Laboratory of Pathophysiology, on a carcinogen-induced mammary tumor system, and is collaborating in the preparation of a manuscript reporting the results.

Dr. Pettigrew is now collaborating with Dr. Guillino on the problem of tumor growth rates and regression rates, as well as advising on the technical question of the best method of estimating tumor size.

Dr. Layard consulted with Dr. William R. Levis, Dermatology Branch, on the analysis of quantitative correlation between in vitro lymphocyte transformation to dinitrochlorobenzene (DNCB) and skin test reactivity to DNCB. For Dr. Walter G. Klingler of the Dermatology Branch, Dr. Layard assisted in the statistical planning and analysis of an experiment involving comparison under various conditions of the activity of ribo-nucleic protein extracted from cow-hoof epidermis.

For Dr. Guy Bonnard who is a visiting scientist in the Immunology Branch, NCI Dr. Connor is analyzing data from a study on cell mediated lysis and the fetal-maternal relationship in man.

Dr. Layard advised Dr. J. Wunderlich and Dr. Israel Yust of the Immunology Branch on the statistical analysis of data from an experiment on the identification of lymphocyte dependent antibody in sera from multiply transfused patients.

Dr. Layard performed analyses of complex factorial experiments conducted by Dr. R. Schwartz and Dr. G. Shearer of the Immunology Branch involving the stimulation of DNA synthesis by antigen in immunized responder and non-responder mice.

Dr. Pettigrew has consulted with Dr. Wells, Dr. Pilch and Ms. Wexler of the Surgery Branch on a variety of topics ranging from clinical studies to murine mammary tumor systems.

Dr. Connor and Dr. Pettigrew have been serving as members of the Ad Hoc Committee to consider responses to an RFP for a Central Statistical Group for a Collaborative Study in Early Lung Cancer Detection Screening. This committee, under the direction of Dr. William Pomerance, Chief of the Diagnosis Branch, DCBD, has held several meetings, and has made site visits to four of the responding institutions, prior to awarding a contract.

Miscellaneous

For Dr. B. Fox of the OADFS&S, Dr. Layard performed several analyses of data in a study done by a University of Virginia research group (Abse et al) titled "Personality and behavioral characteristics of lung cancer patients."

For Mr. Scotto of the Biometry Branch, Dr. Gart developed methods for comparing ratios of incidence rates for a skin cancer survey. Mrs. Smith implemented this method with a computer program.

Dr. Pettigrew is now working with Dr. Wells, now at the Duke University Medical Center, on the preparation of a manuscript reporting the results of attempts to transplant parathyroid glands in dogs.

Dr. Connor provided statistical assistance to a variety of NCI scientists; these include Dr. Charles Litterst of the Laboratory of Toxicology, Dr. Giuseppe Fossati, Dr. Robert Oldham, and Dr. Holgar Kirchner of the Laboratory of Cell Biology, Dr. Howard Dickler of the Immunology Branch, and Ms. Betty Jo Fowlkes of the Laboratory of Pathology.

Dr. Connor served as a statistical consultant to some scientists outside NCI recommending experimental designs and/or specific statistical methods. These scientists include: Dr. L. Friedman, American Red Cross Blood Research Laboratory, Mr. J. McCoy, Bionetics, and Mr. M. Shapiro, DCRT.

Mr. Thomas served as the section' s expert on high speed computing and during the year consulted with every member of the section on many projects which depend on computers. Several useful new computer routines were added to the sections library this year.

Mrs. Alroy M. Smith continues to play an important supportive role in the data analysis of this section particularly in writing and implementing statistical analyses on the high speed computers.

Significance of the Problem to the Institute:

Members of this section are assuming an essential role in much research within the NCI. Their activities include not only statistical analysis but also in planning valid experiments.

Proposed Course of the Projects:

Several of the projects mentioned in the Major Findings section will continue. In particular the collaboration with the various projects in the Carcinogenesis Area, the association with the tobacco painting research, the Cancer Control Breast Cancer Demonstration Program, as well as other projects are expected to continue.

Honors and Awards

Dr. Gart completed his term as a member of the Biometric and Epidemiological Methodology Advisory Committee of the FDA.

Dr. Gart continued to serve as a member of the Biometry Panel of the Scientific Advisory Board of the National Center for Toxicological Research, Pine Bluff, Arkansas.

Dr. Connor served as a referee for Science, Mr. Nam, Dr. Gart and Dr. Connor served as referees for The Journal of the National Cancer Institute. Dr. Layard and Dr. Gart served as referees for Cancer Research.

Publications

Harada, M., Pearson, G., Pettigrew, H., Redmon, L., and Orr, T.: Enhancement of normal lymphocyte cytotoxicity by sera with high antibody titers against H-2 or virus-associated antigens. Cancer Research, 33,2886-2893, 1973.

Lavrin, D.H., Rosenberg, S.A., Connor, R.J., and Terry, W.D.: Immunoprophylaxis of methylcholanthrene induced tumors in mice using Bacillus Calmette-Guerin (BCG) and methanal extracted residue (MER), Cancer Research, 33, 472-477, 1973.

Serial No. NCI-4207
1. Biometry Branch, OADFS&S Division of Cancer Cause and Prevention
2. Mathematical Statistics and Applied Mathematics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Research in Mathematical Statistics and Applied Mathematics

Previous Serial Number: Same

Principal Investigators: John J. Gart, Robert J. Connor, H.W.J. Layard, Hugh M. Pettigrew, Donald G. Thomas

Other Investigators: Jun-mo Nam, Alroy M. Smith

Cooperating Units: None

Man Years:

Total:	4.0
Professional:	3.5
Other:	0.5

Project Description

Objectives:

To conduct basic research in mathematical statistics, probability and applied mathematics; to develop new statistical methodology which is especially appropriate to bio-medical sciences.

Methods Employed:

The methods employed are the modern theories of mathematical statistics, probability and applied mathematics. High speed electronic computers are often used to compute appropriate mathematical tables and test approximations by simulation techniques.

Major Findings:

The Research of the members of this section covers a wide spectrum of topics in mathematical statistics, probability and applied mathematics. These are summarized below.

1. Analysis of Ratios and Cross-Product Ratios of Poisson Variates.

John J. Gart has considered exponential models for analyzing ratios and cross-product ratios of Poisson variates, both in the simple and stratified cases. The methods may be applied to the incidence of relatively rare diseases in large populations where the Poisson model may hold for the distribution of new cases. The analyses of stratified cross-product ratios are seen to be analogues of the noncentral distribution associated with the analysis of the common odds ratio in several 2 x 2 tables (Gart, Biometrika, 57, 471-475.) The results of the investigation will be given as an invited paper at a forthcoming national meeting of the statistical societies . Alroy M. Smith has written computer programs by which these methods may be easily applied. This program has been made available to other parts of the Biometry Branch.

2. Tests for Equality of Covariance Matrices.

Maxwell W. Layard used simulated sampling methods to investigate the small sample properties of robust tests for equality of covariance matrices. A paper based on this research has been submitted for publication.

3. Grouping and Testing for Trend in Categorical Data.

Robert J. Connor finished study reported on last year on grouping for testing trends in categorical data with small samples. The Monte Carlo results indicated that asymptotic optimal grouping rules were not of value in small samples (i.e. that equiprobable grouping was comparable).

4. Models and Methods in Gel Electrophoresis.

Hugh M. Pettigrew has continued to develop models to account for various phenomena associated with the use of gel electrophoresis as a technique for estimating the relative amounts of various isomers of enzymes in tissue homogenates. The models are being tested by experimentation in collaboration with Dr. Gerald Clamon of the Lung Cancer Branch, NCI and may lead to improved design of gel experiments. Appropriate statistical methodology for the analysis of the resulting data will be developed. If the isozyme patterns in various normal and

cancerous tissues can be characterized sufficiently well, then it will be possible to develop methods to test a given tissue homogenate, e.g. from organ culture, for presence of tumor cells.

5. Estimating Gene Frequencies in the HL-A Antigen System.

Jun-mo Nam and John J. Gart have been investigating the extension of Bernstein' s methods for estimating gene frequencies (developed for the ABO bloodgroup) to the general case of several alleles. This extension is important for estimating the gene frequencies in the HL-A system, which may be related to the incidence and survivorship of certain types of cancer. Among the results already derived are: (1) the "improved" Bernstein method is not fully efficient in the general case, although it is in the special case of the ABO system, and (2) The "improved" Bernstein estimator is not, in general, necessarily more efficient than the simple "unimproved" estimator. Tables giving efficiencies of the various methods relative to the maximum likelihood estimators are in preparation. Associated tests for fit of the Hardy-Weinberg law are being investigated. Papers based on these results are being prepared for publication.

6. Exact Tests in Bioassay.

Donald G. Thomas has developed efficient computer programs to perform an exact test for trend in an m-strata bioassay. Also, an exact test for equality of slopes and equality of intercept in two sample bioassay problems.

7. Approximate tests for Trends in Bioassay Data.

John J. Gart is investigating approximate tests for non-zero slopes, equality of non-zero slopes and intercepts in analysis of proportion data in one or several strata. The methods will prove useful in analyzing certain carcinogenesis experiments particularly when the numbers may be too large to use the exact methods.

8. Tables for Comparison of Proportions.

Donald G. Thomas and John J. Gart have prepared tables of exact confidence limits for odds ratios and P values for a wide range of possible outcomes of experiments comparing proportions. These tables may be used in deciding the appropriate sample sizes to be used in comparison of proportions in carcinogenesis screening or clinical trials.

9. Models for Mammary Tumors in Rodents.

Hugh M. Pettigrew has continued his efforts to develop models for mammary tumor systems in rodents, and is considering extensions of existing statistical theory that may be necessary to evaluate data from such systems.

10. Bayesian Analysis of Multiple Comparison Procedures.

Maxwell W. Layard is considering the implications of a Bayesian approach for methods of simultaneous statistical inference. D.B. Duncan has investigated the case of an additive loss function; it is proposed to examine the effects of assuming a non-additive loss function on Duncan's conclusions.

11. The "variance" test for Truncated Poisson Distributions.

John J. Gart has completed his investigation of the "variance" test of the Poisson distribution in the case of truncated samples. The results are expressed in terms of ratios of generalized Stirling numbers. The methods are useful for testing the fit of data (radioactivity or other counting data) when the zero reading, for instance, is not observable or relevant. A paper based on these results has been submitted for publication.

12. Detection of Cyclic Trend.

Jun-mo Nam is continuing his investigation of the proper statistical methods to identify the cyclic trend. These methods, for example, may prove very useful to detect a seasonal effect on disease incidence.

13. Censored Rank Order Tests and Survival Curves.

Donald G. Thomas has improved the existing programs for Breslow's rank order tests and added to plots of the appropriate life table results. This program is very useful in analyzing chemical carcinogenesis experiments.

14. Estimation of the Change-point of the Mean of a Normal Distribution.

Maxwell W. Layard is studying the problem of estimating the (single) point at which the mean of a normal distribution changes in response to changes in an explanatory variable. A possible application of this research is in the analysis of dose response data.

15. Nonparametric Tests of Survival Experience with Fixed Point Censoring.

Dr. Maxwell W. Layard and Robert J. Connor are working on methods for analysis of data from carcinogenesis experiments. One aspect considered is a simple test to compare the survival experience with several groups and censoring occurring at a fixed point.

16. Estimation of a Linear Regression when both Variables are Subject to Error.

Maxwell W. Layard is investigating the large-sample properties of normal-theory maximum likelihood estimates under departures from the normality assumption. Approximate mean squared error comparisons of maximum likelihood estimators with ordinary least squares estimates have been made.

17. Models for DNA Synthesis.

Hugh M. Pettigrew, with Dr. C. Wesley Dingman, is applying the theory of stochastic processes to the development of models to explain phenomena associated with DNA synthesis and breakage.

Significance to the Program of the Institute

The interplay between mathematical theory, data analysis and experimental research is an important element in biomedical research. Many of the "Major Findings" reported above are new statistical techniques which have or may be directly applied to data collected by the medical researchers at NCI or other workers on problems in cancer research. Others are mathematical models which may elucidate the biological phenomena involved in cancer research and which may also aid in the planning of subsequent experiments or epidemiologic studies. The opportunity for initiating fundamental research on mathematics and mathematical statistics is essential for enabling members of the section to achieve professional recognition among their peers in their own scientific disciplines. More importantly, the possibility of doing such unconstrained research is a prerequisite for the consulting work of the section to be carried out at the highest professional level.

Proposed Course of the Project:

Many of the projects described in major findings will be continuing, e.g. such as models and methods for various bioassay systems and exact and approximate analyses of counting data. Other problems, not referred

to above, shall also be attacked in the upcoming year; they will include the development of new statistical methods and mathematical models in various biomedical problems, particularly in the area of models for the analysis of survival curves in carcinogenesis experiments.

Honors and Awards

John J. Gart was elected to the Nominating committee of American Association for the Advancement of Science (Section U: Statistics) for 1974-75.

John J. Gart recently completed his two year term as an associate editor of The American Statistician.

John J. Gart was an invited lecturer at the Mayo clinic.

John J. Gart continued to serve as an elected member of the Regional Committee of the Biometric Society.

Maxwell W. Layard served as chairman of a contributed papers session at the Annual Meeting of the Biometric Society at New York City in December 1973.

John J. Gart was appointed the American Statistical Association Representative on the Regional Committee of the Biometric Society.

Robert J. Connor and John J. Gart served as referees for The Journal of the American Statistical Association. Donald G. Thomas and Robert J. Connor refereed for The American Statistician. John J. Gart refereed for The American Journal of Epidemiology.

Publications

Arvesen, J.N. and Layard, M.W.J.: Asymptotically robust tests in unbalanced variance component models. Annals of Statistics, in press, 1974.

Duncan, G.T. and Layard, M.W.J.: A Monte-Carlo study of asymptotically robust tests for correlation coefficients. Biometrika 60: 551-558.

Gail, M. and Gart, J.J.: The determination of sample sizes for use with the exact conditional test in 2 x 2 comparative trials. Biometrics 29: 441-448, 1973.

Gart, J.J.: A review of the theory and application of methods for the comparison of proportions. Proc. of the Seventeenth Conference on the Design of Experiments in Army Research and Development and Testing. U.S. Army Research Office - Durham, N.C., ARO-D Report 72-2, 1972 Part 2: 1007-1017.

Greenstreet, Richard L. and Connor, Robert J.: Power of Tests for the Equality of Covariance Matrices. Technometrics 16: 27-30, 1974.

Serial No. NCI-4268
1. Biometry Branch, OADFS&S
   Division of Cancer Cause and
   Prevention
2. Special Cancer Survey Section
3. Bethesda, Maryland

NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Third National Cancer Survey

Previous Serial Number: Same

Principal Investigators: John Young and Sidney J. Cutler

Other Investigators: Paula Baylis, Roger Connelly, Susan Devesa Harvey Geller, James Larson, Constance Percy, Joseph Scotto, Theodore Weiss, Roger Williams

Cooperating Units:
University of Alabama School of Medicine
Georgia Regional Medical Program
State of Colorado Department of Health
University of Texas Southwestern Medical School
Michigan Cancer Foundation
University of Iowa
Mayo Clinic
University of Minnesota, Department of Epidemiology, School of Public Health
University of Pittsburgh, School of Public Health
Puerto Rico Commonwealth Health Department
State of California, Department of Public Health
Iowa State University
Epidemiologic Pathology Unit, NCI
Electronic Data Processing Staff, BB, NCI

Man Years:

Total:	5
Professional:	2
Other:	3

Project Description

Objectives:
To prepare a complete register of all new cancer cases diagnosed in the resident population of seven standard metropolitan areas, two states, and the Commonwealth of Puerto Rico during the period 1969-71, plus all other cancer cases seen by doctors or hospitals

in those areas; to obtain demographic data and a description of the tumor for each patient; and to study certain economic, social, and other aspects of the disease in a sample of patients.

Methods Employed:

In each survey area, NCI contracted with local organizations to hire field staff and gather data. Data were requested from doctors and obtained from hospital records and death certificates. With approval of their doctors, a sample of cancer patients were interviewed. Local staffs did the preliminary editing and coding of data. Institute staff in Bethesda, Maryland, completed the editing and coding of data and converted it to magnetic tape for use in preparing detailed reports through electronic computers.

Basic information was being collected on every active cancer case, except certain skin cancers, during the three-year period. This included race, age, place and date of birth, sex, and marital status. Medical information included the primary site and histologic type of tumor, date and method of diagnosis. Information available from any existing cancer register or continuing survey in a survey area was utilized to avoid duplication of effort. All reports were treated as strictly confidential.

Death certificates mentioning cancer were obtained from the appropriate vital statistics offices. Local survey staff or hospital personnel abstracted information about cancer patients from hospital records.

Trained interviewers, after receiving written permission from attending doctors, obtained from a selected sample of patients information on the amount of disability incurred, time lost from productive activity, cost of treatment, and sources of payment. Questions were also asked regarding religion, number of children, socioeconomic status, and personal habits such as smoking and drinking. Hospital records on this selected population were reviewed for more detailed information on cost of hospitalization, methods of meeting this cost, treatment for the cancer, and the extent of the disease at time of diagnosis.

Workshops were conducted periodically to resolve problems which the supervisors had encountered. The format of round table discussions on specific questions and informal discussions among the supervisors proved very effective in carrying out the instructional and communicative objectives of these workshops.

Extensive use has been made of computer capabilities in the processing of the survey documents. Each document was processed separately, with the computer tagging and combining all the records for an individual patient. The computer conducted a large number of edit checks--consistency among documents on the same patient, as well as among items on the same document. The sample selection was also a computer operation with the printing of the appropriate labels included in the operation. The data

processing time was reduced to a minimum. Within five weeks after a record was received at NCI, the data were converted on to computer tapes, edit runs were completed, and printouts of error reports and updates of survey control reports were mailed back to the field offices. Data were accessed on a weekly cycle and collated into the master file. Each item was reviewed to insure that it was within set limits and that the coded information was a valid code. Other edits included determination of consistency or validity between items on the same abstract form. The computer also checked for consistency in data among the various forms on the same patient. There was a feedback to the survey areas of any invalid, inconsistent, or questionable data. The survey field offices researched the problems and corrected the data stored in the computer tapes. The data were reviewed periodically to insure that corrective action had been taken by the survey field office.

As the survey approached the completion of the data-gathering operation, a variety of reports were being produced to assist in assurance of complete reporting and accuracy of the information. These included (1) listing of cases that required follow-back, such as death certificate abstracts, to insure accuracy of the data; (2) listing of cases that required supplemental data on medical care and hospital costs to insure complete reporting of these cases; and (3) special listing of cases with erroneous or questionable data to insure that the cases were researched and corrections made.

Because of limited staff available, some of the quality control procedures were modified. The reinterview program was eliminated because of the problems in obtaining the reinterviews, and the lack of feedback either to the survey field office staff or the NCI. The reabstract program was also modified by eliminating the reabstracting of a random sample.

The last batch of basic records was received in the central office from the fields in November. In January a tape was created which contained data extracted into a single record for each patient from all basic documents ever received on a given patient, except that, if a patient had two or more cancers active during 1969-71, an extract record was created for each case. A similar extraction will be completed for sample data in the near future.

A special skin cancer survey was started September 1, 1971, which collected data on cases diagnosed through February 29, 1972. Only four of the survey areas--Iowa, Dallas, Minneapolis, and San Francisco--are participating in this special study. Special efforts were made to contact all physicians who would see or treat skin cancer patients to obtain their cooperation. Pathology laboratory files supplied data on cases that were histologically diagnosed. In addition, abstractors completed separate forms for skin cancer patients found during their normal visits to

participating hospitals. Data collection has been collected and magnetic tapes have been created to use in data analysis.

Major Findings:

An advanced report based on the 1969-1971 incidence data was distributed to the Institute staff and principal investigators. Reports specific for each field office as well as all field offices combined are being prepared for wide distribution. The three-year report for all areas combined differed from the 1969 Preliminary Report in two major respects: (1) data for all of Colorado rather than the Denver SMSA were included, and (2) *in situ* carcinomas were excluded from every site. Neither report contained data from Puerto Rico.

The advanced report showed that from 1969-1971 there were 181,036 newly diagnosed cancers in the survey areas (population in 1970; 21,003,451) yielding an average annual incidence rate of 300.0 per 100,000 age-adjusted to the 1970 United States population. This rate corresponds to 610,000 new cases diagnosed annually in the total United States. The age-adjusted rates for males was considerably higher than for females, 346.8 vs. 270.2 per 100,000 respectively. Negroes had higher age-adjusted rates than whites, 319.0 vs. 297.7 per 100,000 respectively. Markedly higher rates among black males were noted in the prostate, esophagus and lung. Black females and higher rates for cancer of the cervix while white females had higher rates for cancer of the breast and cervix.

While the areas covered by the present survey are not exactly the same as the areas covered in the survey of 1947, a number of trends are worthy of note. Adjusting the rates to the United States 1950 population, the incidence rates among males increased from 280 in 1947 to 305 in 1969-1971, while the rates among females decreased from 294 to 252 for the same time periods. The increase in the male rate is due largely to substantial increase in the incidence of cancers of the lung and prostate, and a somewhat lesser increase in incidence of colon cancer. These increases more than counterbalanced the drop in incidence of gastric and rectal cancers. The decrease in female rate is the result of a drop in the incidence of cancers of the cervix uteri, stomach, and rectum.

The data also showed that among the survey areas cancer of the lung, prostate, digestive tract, breast, and uterus demonstrated geographic differences.

Findings from the special skin cancer survey indicated that the estimated incidence of the site is grossly understated. Even in the survey area with the lowest incidence rate tabulations indicated more than twice the number of cases than expected. Because of this large increase of cases over expected, the data were reviewed with a number of consultants including a committee

appointed by the National Academy of Science at the request of the Institute.

A booklet on cancer incidence, survival and mortality for children under 15 years of age was prepared in collaboration with the American Cancer Society for distribution as a professional education publication at a National Conference on Childhood Cancer in May, 1974. From 1969-1971 there were 1,938 cancers diagnosed in white children who were residents of the Third National Cancer Survey areas yielding an average annual incidence rate of 12.5 per 100,000. Fifty-four percent of these cases were due either to leukemia or tumors of the brain and nervous system. The incidence of neuroblastoma was 0.8 per 100,000.

Listings of individual patients reported by each participating hospital were prepared. For hospitals over 200 beds tabulations by site and year of diagnosis were also prepared. These tabulations and listings have been distributed to the hospital administrators of participating hospitals for use in their hospital registries and medical record libraries. This service has proven to be an excellent public relations procedure and has provided hospitals with useful planning data.

Significance of the Program to the Institute:

Registration of all cancer cases in a defined population is the only way to acquire reliable data on cancer incidence rates. The rates themselves are indicators of the significance of cancers in the various sites or of different types. This data bank will provide a valuable source for epidemiologic investigations by identifying population groups that have a high or a low incidence of cancers of specific sites. Some of the factors that will be studied include residence, occupation, place of birth, use of contraceptive devices, and the use of tobacco and alcohol.

Other products of the survey that will be important to the mission of the National Cancer Institute will be studies on the delivery of medical care to a large and representative sample of cancer patients, treatment provided the cancer patient, extent of disease at time of diagnosis, direct and indirect costs of cancer, and the extent and duration of disability from cancer.

The survey has produced unique data which can be used by other investigators as a starting point for more intensive investigations. One area of special interest is rare tumors. Because of the low numbers of these tumors, epidemiologists feel that investigations will yield significant results and possible etiologic leads.

The data contained in the preliminary and advanced reports are a valuable source of information not available anywhere else in the country. Daily, a large number of queries are received on cancer

sites and histology to which we are now able to respond with data. The information has also been useful in program planning and in preparing reports for NCI, NIH and HEW use. The survey data have also indicated there were errors in estimates of incidence by site used by various cancer agencies. The data will enable us also to provide a more sensitive method for determining estimates of the incidence of cancer in the future. Further, the availablity of incidence data has enabled Institute staff to assist other program areas, particularly the Cancer Control Program, in site reviews and contract awards.

Proposed Course of the Project:

The data collection operation has been completed. Final data tapes containing information about the site, type, date and method of diagnosis of all cancers seen in the survey areas between 1969-1971 have been created. These tapes have been used to produce the three-year advanced report and the hospital tabulations mentioned above. At present, these tapes are being utilized to produce an NCI monograph containing detailed incidence data by race, sex, age, geographic area, site and type of cancer. The information of incidence by histologic type is much in demand and it will answer many requests for such data being received daily by Institute staff. The monograph should be published by the end of the fiscal year.

A core staff remains in each of the field offices. These persons are being utilized to distribute data as it becomes available to hospitals, physicians and other appropriate groups within their own local areas. The core staff are also assisting Institute staff in assigning census tracts to each of the newly diagnosed cases for use in further analysis. In some instances core staff are participating in a local analysis of data.

No field office will be kept open beyond August 31, 1974. It is the responsibility of each of the field offices to dispose of their original records. Three of the field offices - San Francisco-Oakland, Detroit, and Iowa - have already been absorbed into participating SEER programs and a fourth - Atlanta - may be by the end of the fiscal year.

In addition to the monograph containing detailed site-type information for all areas combined and each specific area, two other monographs are planned for the near future. These monographs will contain data on socioeconomic status, prevalence, length of hospital stay, medical costs, cancer among the Spanish population, stage at diagnosis, medical treatment, geographic variation and detailed data on the incidence of non-melanoma skin cancer. Also, Institute staff are preparing papers on individual cancer sites, cancer among children and neonates, cancer among various racial groups, and the accuracy of cancer reporting on death certificates.

The interview data still requires attention. Portions of the interview appear to be complete, and the data seem valid. The procedure for data conversion is being investigated by the systems analysts and should be on computer tapes in the next year. A staff member has been assigned to make recommendations on the approach that should be taken in analyzing interview data, i.e., should analysis be restricted to certain cancer sites or certain geographic areas. Because of staff limitations the interview has had low priority but more progress is expected during the next year.

An Advisory Committee for Utilization of Data from the Third National Cancer Survey is being established and will assist the staff in assigning priorities to future analysis and in handling requests for data by outside research groups.

Honors and Awards:

Lectures:

Joseph Scotto "The Incidence of Non-melanoma Skin Cancer in Four Geographic Areas of the United States" presented to the Climatic Impact Assessment Program of the Department of Transportation, Gainsville, Florida, 1973.

Constance Percy "The Incidence of Cancer in the Dallas-Ft. Worth SMSA, 1969-1971" to be presented at the Texas State Department of Public Health Workshop for Tumor Registrars, Austin, Texas, April, 1974.

John Young "Trends in Cancer Morbidity and Mortality" presented at the Florida State Department of Health Workshop for Tumor Registrars, Miami Beach, Florida, September 24, 1973.

"The Incidence of Cancer in the United States" presented at the University of California Medical Center, Tumor Registry Training Programs November 9, 1973 and February 15, 1974.

"Trends in Cancer Morbidity and Mortality in the United States with Special Attention to Breast Cancer" to be presented at the New York State Department of Public Health Workshop, Albany, New York, April 24, 1974.

Sidney J. Cutler "Geographic Variation in Cancer" presented to the Washington Statistical Society, Washington, D.C., February 15, 1974.

Consultants:

Joseph Scotto - Consultant to Climatic Impact Assessment Program - Subcommittee on Effects of the SST.

Committee Memberships:

Roger Connelly - Member of Working Cadre of the National Program - Pancreatic Cancer.

Committee Memberships: (continued)
Constance Percy - Member of the United States Working Party for the 9th Revision - International Classification of Diseases.

Publications:

Advanced Three Year Report, Third National Cancer Survey, 1969-1971 Incidence, February 1, 1974.

Cancer in the Atlanta Metropolitan Area, Third National Cancer Survey, Advanced Three Year Report 1969-1971 Incidence, February 1, 1974.

Cancer in the Birmingham Metropolitan Area, Third National Cancer Survey, Advanced Three Year Report 1969-1971 Incidence, February 1, 1974.

Cancer in Colorado, Third National Cancer Survey, Advanced Three Year Report 1969-1971 Incidence, February 1, 1974.

Cancer in the Dallas-Ft. Worth Metropolitan Area, Third National Cancer Survey, Advanced Three Year Report 1969-1971 Incidence, February 1, 1974.

Cancer in the Detroit Metropolitan Area, Third National Cancer Survey, Advanced Three Year Report 1969-1971 Incidence, February 1, 1974.

Cancer in Iowa, Third National Cancer Survey, Advanced Three Year Report 1969-1971 Incidence, February 1, 1974.

Cancer in the Minneapolis-St. Paul Metropolitan Area, Third National Cancer Survey, Advanced Three Year Report 1969-1971 Incidence, February 1, 1974.

Cancer in the Pittsburgh Metropolitan Area, Third National Cancer Survey, Advanced Three Year Report 1969-1971 Incidence, February 1 ,1974.

Cancer in the San Francisco Bay Area, Third National Cancer Survey, Advanced Three Year Report 1969-1971 Incidence, February 1, 1974.

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1. Biometry Branch, OADFS&S, DCCP
2. Automatic Data Processing Management Section (ADPMS)
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Data and Information Processing Consultation and Assistance

Previous Serial Number: Same

Principal Investigator: Theodore Weiss

Other Investigators: Altamease A. Gales, Terence D.C. Kuch, James E. Larson, Norman Oslik, J. Michael Stump

Cooperating Units: None

Man Years :

Total : 10
Professional: 5.5
Others : 4.5

Project Description

The Automatic Data Processing Management Section provides consultation and assistance to the scientific and administrative staff of the National Cancer Institute. It has expertise in applying data and information processing technologies to individual as well as to cooperative epidemiologic, laboratory, and clinical research projects, program planning and appraisal activities, and administrative management operations.

Objective:

To further the research effort through the appropriate application of computer science and information technology; and through minimizing the mechanical and logistical constraints upon investigators arising from scientific investigations which involve data and information collection, conversion to machine-readable form, purification, computation, storage, retrieval, analysis, and dissemination.

Methods Employed:

Consultation and assistance is provided to research projects in three disciplinary areas:

Planning and Appraisal: participating in the planning and evaluation of research projects which have data and information processing activities; assisting investigators and their research contractors develop sources of computer-related services; conducting studies and disseminating information on the feasibility of using computer-related techniques, such as optical scanning versus key-to-disk or keypunching for data capture, computer-related generalized proprietary software packages versus custom-designed programs and systems, and computer-related equipment, such as on-line communications terminals; and developing standards of quality and performance of data and information processing products and services provided by research contractors, the ADPMS, and commercial ADP contractors;

Information Systems Development and Statistical Computing: providing technical assistance through forms design, systems analysis, systems design, the preparation of reference and procedure manuals, and computer programming services using digital computers, scientific computers with analog-digital converters, and related electronic instrumentation; providing generalized software systems and programs for investigators' own use; and conducting workshops and individual instruction in the use of these programs and systems; and

Operational Monitoring and Control: coordinating and monitoring the conversion of raw data into forms appropriate for computer processing and the day-to-day operation of data processing systems and activities; and maintaining and operating a Remote Job Entry Terminal Facility with such equipment as the IBM 2922.

Major Findings:

ADP Management Section

This has been a significant year for the professional staff of the Section. Over the past few years there has been an ongoing transition of the staff from data processors to biomedical computer systems specialists, accomplished by a shift away from the traditional data processing methods used in the non-scientific environment, and toward greater involvement in the larger problems of biomedical information systems and statistical computing. Most of the professional staff of this Section, initially without backgrounds in biomedical research or collaborative methods, have increased their expertise in these areas to become more effective in contributing to the investigations conducted by the Biometry Branch, the Field Studies and Statistics Area, other areas of NCI, and on occasion, institutions engaged in other areas of medical research.

Several years ago when punched cards were the primary storage medium for data and access to computers was often slow and frustrating, individual and cooperative studies were generally modest in size, cost, and complexity. With the dramatic growth of funding and scope of research programs, and the development of complex computer hardware and sophisticated software, the need for the biomedical computer systems specialist was recognized by the Biometry Branch. This Section was established three years ago to meet these requirements.

As with most new fields, there were very few individuals who could be considered "prepackaged" to meet these needs. For this reason a small number of experienced computer specialists were recruited who were willing to continue their education in the biological sciences, medicine, and statistics. Their continuing education and experience has given the staff the necessary background to understand the disciplines and methodologies appropriate for consultation in the Institute. Enough of the transition has been completed to where the staff are now engaged in a broad range of computer-related consulting activities in many areas of cancer research.

There have been a number of accomplishments this fiscal year toward improving the library of data management systems and statistical computing programs available as generalized investigative instruments; toward improving the accessibility and capability of data processing equipment and services; and toward improving the content and delivery of information to Program Directors for planning, budgeting, and evaluating their computer costs and services.

The Generalized Reporting Program (GRP) obtained by the Section continued to receive widespread use within the Biometry Branch. GRP is a software package which, with a few instructions, allows investigators to write their own computer programs to produce selected lists and tables. The ADPMS has received requests to modify GRP to give it the capability of adding population data and producing incidence rates as well as to create selected subsets of large data bases for more economical analysis of the data, and to enhance the documentation package so that the system can be used by investigators at other institutions involved in the Branch's research program.

Installation of the Conversational Computing Statistical System, CCSS, jointly supported by ADPMS and the Epidemiology Branch, NICHD, was completed on the PDP10 system during the year. CCSS is a system used to edit, input, calculate, and retrieve statistical data. It was originally developed by the Department of Biostatistics at the University of Washington. Mr. Stump serves as the principal data processing consultant representing ADPMS. System modifications have been defined and are in the process of being implemented to make the system operational on the IBM 360/370 computers. Implementation on the IBM hardware is being accomplished to remove several system constraints, such as limited disk space experienced by users of CCSS on the PDP-10, and to allow data sets to be exchanged and used within the larger IBM program library.

A remote Job Entry Terminal Facility has been operating under the direction of the ADPMS. The Facility contains an IBM 2922 (Remote 17) with card reader and high speed printer, several IBM 2741 communication terminals, a keypunch/card interpreter, and a sorter-counter. A production desk-type service is operated by the ADPMS. In addition, a central Landow-DCRT pickup and delivery service operates from the Facility. Remote 17 is funded jointly by the National Heart and Lung Institute and the National Cancer Institute.

The Field Studies and Statistics Area

The ADP Management Section provided consultation and assistance to five Biometry Branch projects during the year:

The Third National Cancer Survey

The Third National Cancer Survey has been the primary focus of this Section's consulting activities for the past five years. The Study's methodology is largely incorporated into a complex computer system which was designed, programmed, and operated for four years by this Section and its contractors.

Mr. Theodore Weiss developed the overall plans for the data processing system and designed the data collection forms in the Fall of 1967. He participated in the design and implementation of the field offices' manual files and procedures and directed the detailed design, programming, and early operation of the Study's computer system. Mr. Terence Kuch designed the data keying and optical scanning portion of the system. Mr. James Larson has coordinated the daily operation and ongoing development of the data processing system for the past three and one-half years.

This year Mr. Larson planned and directed a major portion of the data management work required to close the Study and prepare the data base for analysis and publication.

Over one million abstract documents for 350,000 cases of cancer were handled by the Survey's data processing system during the four-year data gathering phase. A separate computer system was developed this year to assure that all abstracts were accounted for and that all inconsistencies were corrected. The data base was then converted from the format used for data gathering to one more appropriate for analysis.

The computer programs developed for the conversion process were particularly complex, especially the routines which determine the number of independent primary cancers for a case and the anatomical site, histologic type, and malignancy of each neoplasm.

Several hundred statistical tables were calculated and printed in camera-ready form for the publication of the Third National Cancer Survey, Advanced Three Year Report, 1969-1971 Incidence.

Subsequently an NCI Monograph on cancer incidence was produced using a computer system which calculated and formatted all of the statistical tables and then made offset printing plates directly from magnetic tape. This technique saved both time and money by eliminating the considerable effort usually required to set type manually and to proofread voluminous statistical tables.

Detailed geographic coding of cases was accomplished on the computer for the investigation of variations in incidence rates by socioeconomic factors. The U.S. Census Bureau's program, UNIMATCH, and extensive manual work by the field offices determined the census tract designation of all cases residing in standard metropolitan statistical areas. Cases living outside of metropolitan areas in two states were classified as urban or rural residents by a computer program developed for this purpose.

Considerable effort has been devoted to the orderly closing down of the Study's data processing system. All computer programs have been documented and stored in an appropriate manner. All other processing materials, such as computer tapes and listings, data collection forms, and operation manuals, have been reviewed for final disposition and, where appropriate, placed in long-term storage. These steps, although time consuming, are critical to conform to the confidentiality requirements of the Study, to provide documentation for further analysis of the data, and for future surveys.

It is expected that further consultation will be required over the next several years; but it will be at a lower level of effort than before, and of a different nature. The past five years have involved primarily operational planning, systems analysis, data management, and contract coordination and review. Future consultation on the Survey will be primarily statistical computing in nature.

Technical consultation will be provided for the two monographs scheduled for the coming year. These monographs will contain data on medical costs, cancer among the Spanish population, stage at diagnosis, medical treatment, geographic variation, the incidence of non-melanoma skin cancer, and numerous other special studies.

The capture and analysis of patient interview data will require data management and analysis consultation. A statistical computing software package will be developed to facilitate data analysis.

Much of the expertise and many of the techniques developed for the Third National Cancer Survey are applicable to the SEER Program. As the Survey's requirements decline, an increasing proportion of the staff's consulting time is being directed to the SEER Program.

The data collected for the Survey provides a valuable source for epidemiologic investigation, for the planning of cancer treatment facilities, and for the evaluation of cancer detection and treatment programs.

Without the benefit of the Survey's computer system, the availability of this critical data would have been delayed for many years. Complex edits performed by the computer as abstracts were received from the field, and sophisticated computer-based extract, calculation, and reporting techniques have greatly enhanced the investigators' perspective of the data and reduced the time needed to produce statistical tables for analysis and publication.

A Preliminary Report of 1969 Incidence was published only six weeks after the field offices completed 1969 case findings, an Advanced Report of Three Year Report, 1969-1971 Incidence was published two months after the completion of data gathering, and all statistical tables for the NCI Monograph on cancer incidence were available four months after completion of data gathering.

In conclusion, much of the scope, schedule, expectations, and overall success of the Third National Cancer Survey are a direct product of the systems analysis and design consultation provided by this staff and its contractors.

The Surveillance, Epidemiology and End Results Reporting Program (SEER)

Several members of the Section began extensive consultation and support of the SEER Program this year. (For a description of the research project see SEER Project Report, Biometry Branch, NCI.) This program is broader in scope and more complex in nature than the End Results Program. Conceptually, it embodies much of the experience, techniques, and methodology learned from the End Results Program and the Third National Cancer Survey.

Mr. Weiss is a member of the SEER Coordinating Committee, which is responsible for the overall organization and coordination of all aspects of the startup phase of this new program. Specific kinds of activities include the development and implementation of the plans, schedules, coding schemes (where appropriate), operational guidelines, and submission requirements by the program's participants, as well as in the organization of training sessions, workshops, and conferences. Mr. Weiss has made site visits to a number of the participating Registries to consult with them as to their data processing and operational requirements. This has been done to assist the Registries in determining whether their existing computerized systems can be modified to support the SEER Program (if the Registry has been part of the End Results Program), or whether new systems would be more appropriate, and as for newly established Registries, to determine what approaches might best be used to obtain the systems design and computer programming services and computer facilities required to develop, implement, and operate new computerized systems for the program.

For Registries converting from the End Results Program to the SEER Program, this usually involved extensive discussions with the Registry's staff, detailed technical reviews of the existing computerized systems, procedures, manual (paper) and automated files, data conversion (keying) activities, quality control procedures in data processing (computer editing), and security and confidentiality measures. This review considered the implications of implementing a new system on the Registry's ongoing operations, the conversion of their files, the rewriting of procedures, etc. The Registry's workloads, data processing budget, and actual expenditures for data processing services were also studied. Recommendations were made to the Registry concerning these matters.

Mr. James Larson and Mr. Michael Stump made site visits to several Registries in the SEER Program to serve as consultants and advisors to the Registries during this startup period to assist them in the technical planning of their new computerized systems. They participated in the design of the new systems, coordinated the efforts of the Registry staff with those of the systems analysts and computer programmers, and provided the technical review necessary for such an effort to assure that the work was consistent with the requests of the Registry and with the SEER Program.

A number of other significant consulting activities took place during the year and a variety of important materials were being developed for the Program. Mr. Stump implemented a generalized table-making and data retrieval system at several registries. This system was installed by the ADPMS for Biometry Branch investigators and SEER Program participants.

Mr. Terence Kuch began the compilation of an indexed collection of printed materials, with emphasis on materials outside the journal literature, in the specific area of cancer registries such as case finding, follow-up, abstracting, analytic versus non-analytic handling of disproven cancers, etc., and in supporting areas such as statistical computer systems, coding of pathology findings, and record-linkage.

It is envisioned that this collection will grow into a library of general use to Branch staff members and SEER participants around the country.

Mr. Kuch and Mr. Weiss are designing general specifications for a computerized cancer registry data processing system. This system will enhance data entry, file processing and maintenance, data purification and correction, etc., and should be of sufficient power and generality to be directly applicable to most cancer registries, and at least helpful to the others. This general model of a computerized Tumor Registry System will not actually be completed by the ADPMS in terms of computer programming because the programs would depend upon the particular equipment, software, and programming expertise available in each registry. Mr. Kuch and Mr. Weiss are contributing to the portion of the SEER Program Handbook which relates to data processing.

The Japan-Hawaii Cancer Study

The Japan-Hawaii Cancer Study was begun in 1971 to do interpopulation and case-control studies involving Japanese and Caucasians in Hawaii and Japanese in Japan. (See Japan Hawaii Cancer Study Project Report, Biometry Branch, NCI for a description of the research project.) Mr. Norman Oslik has been involved with the project since its inception as the principal data processing consultant and systems designer. Over the past year, Mr. Oslik has worked toward achieving a smoothly operating data processing system in Honolulu for the JHCS. The computer system that was initially developed in Bethesda by Wolf Research and Development Corporation (August 1971 through March 1973), using the NIPS generalized data management package, has been successfully implemented at the University of Hawaii computing center. Mr. Oslik spent a year in Honolulu transferring the computer aspects of the system, developing manual procedures in the clinic, training clinic personnel, and monitoring data processing subcontracts. He also recruited and trained a systems analyst to take over responsibility for all future systems maintenance.

Presently the system has the capability of adding to and retrieving from the computer file the data from approximately 40 data collection forms, including questionnaires, laboratory results, pathology data, and hospital surveillance forms. During the year a fourth NIPS data file was constructed for all surveillance and pathology data. This supplements the Cancer Master File, the Heart Study File, and the Name and Address File.

Subjects have been coming into the clinic for the Third Examination cycle since October, 1971. All data ascertained through a subject's routine clinic exam, with the exception of certain lab results are entered into the computer file within six weeks after collection. After each monthly update of the file, computer-generated letters containing various test results are produced and sent to the subjects and their doctors. Also, the edit errors detected by the computer are researched and the data corrected using a simple change document that can be coded to correct any item of data.

Progress was made in the collaborative effort with Akita, Japan. Mr. Oslik visited Akita and met with Drs. Kamiyama and Ishidate. They discussed in detail the forms and protocols to be used in the data collection effort and resolved many of the communications difficulties inherent in such an international collaboration.

A subcontract was let to have all documents collected through October 1973 put onto microfilm. All documents which have been filmed are currently being stored in a Honolulu warehouse until it is considered safe to destroy them.

Confidentiality of the data collected is of utmost concern. Various procedures have been implemented to protect the data, such as employee confidentiality pledges, locked record rooms, incineration of unneeded records, and separate name and address computer files.

Some analyses of the Third Exam data, often in conjunction with Heart Study data, have been done using existing statistical programs where available (UCLA BMD, etc.) and writing some new ones.

Some clerical and automated procedures for record linkage have been developed to prevent the same individual from entering the study at different times with different ID numbers. This becomes more critical as JHCS expands its surveillance and as it interfaces with the Hawaii Tumor Registry.

Data processing work will continue in several areas. Maintenance of the JHCS computer system will be an ever present task. This includes the design of new data collection forms and their addition to the computer system, writing edit programs for the new data, correcting programming errors, making refinements to existing programs, and making adjustments required by changes in software or hardware at the University of Hawaii computer center.

New types of computer programs for analysis are being developed as they are required.

The ADPMS will continue to have an active consulting role in this project.

Continuing Demography Studies

The Japanese Migrant Studies and the Study of Cancer Among Retired Railroad Employees received continued systems analysis and computer programming support from ADPMS. Mr. Stump served as the principal data processing consultant on these projects, and Ms. Altamease Gales and Ms. Caren Parsons contributed the major computer programming support. Additional data were added to the Japanese Colon Survey and the Hawaii Lung Survey using modified versions of the JMS edit and update programs. In addition, all of the Japanese Migrant Study files were reformatted so that a single version of the Generalized Reporting Program (GRP) could be used to produce a variety of reports for analytical purposes. A subset containing a 4% sample of the 1.5 million records comprising the Study of Cancer Among Retired Railroad Employees was created and used to further reduce the cost of data analysis. While the major technical efforts of these studies have been completed, minor data processing consultation and programming support is contemplated for some time.

Epidemiology Branch Support

Members of the staff consulted on several projects within the Epidemiology Branch:

Dr. Robert Hoover, Epidemiology Branch, and Mr. Stump are collaborating with an independent investigator concerning the Effects of Estrogen Treatment in Women. Data on patients treated with estrogen for menopausal symptoms have been forwarded to Dr. Hoover for preliminary analysis. Mr. Stump is assisting in the determination of whether there are statistically significant variations in cancer incidence rates between this population and the expected rates in

the general population at risk. Based upon the results of this preliminary analysis, a decision will be made as to whether a more detailed analysis is warranted.

Using computer routines developed by the ADPMS, as well as some used in various other studies, and using incidence rates obtained from the End Results Program/Connecticut Tumor Registry, the expected rates of cancer incidence (by site) were calculated for this patient population. The results of these tests are showing that this data source is a potentially valuable one, and efforts are under way to edit the data more extensively and conduct more extensive and refined analyses. Mr. Stump will continue to participate in this project.

Since 1968 the ADPMS has been providing consultation, computer programming, and operational support to the Epizoology Section's Veterinary Medical Data Program. Originally, this system of computer programs written and used at Michigan State University was modified and transferred to DCRT by Mr. Oslik. The computer system is used to collect, store, retrieve, and analyze pathological and clinical procedure data on patients from fourteen university-affiliated veterinary clinics in the United States and Canada. Over the years the basic system has been further modified by members of the staff to accommodate an expanded data collections form, to incorporate more extensive data edits, to produce additional reports, expedite processing, and reduce operational costs.

During this fiscal year the staff relinquished responsibility for the operational aspects of the Veterinary Medical Data Program to the Epizoology Section, but have continued to serve the principal investigator as consultants to the Program. The nature of ADPMS consultation this year included participation by Mr. Stump in the selection of a new data conversion contractor to key the input abstracts, modification of the computer programs in the system by Mr. Oslik and Mr. Stump, and the attendant changes in documentation necessary for the routine operation of the system at the Division of Computer Research and Technology.

Mr. Kuch assisted Dr. William Priester and Dr. Howard Hayes in the design, writing, and dissemination of the VMDP Procedures Manual, a detailed explanation of VMDP procedures conducted within the Epizoology Section, as a supplement to the existing VMDP Users Manual, which is intended primarily for participating veterinary clinics. Mr. Kuch also consulted on the microfilming of VMDP abstracts showing tumor pathology, and suggested various techniques to simplify portions of the VMDP system. Consultation by members of the staff is expected to continue on this project.

The Carcinogenesis Area

Mr. Kuch consulted with the Carcinogenesis Program, especially the Carcinogen Bioassay and Program Resources Branch and the Information and Resources Segment, the former concerned with determining chemical and physical causes of

cancer through large-scale bioassays of laboratory animals, and both concerned with the development of animal resources, chemical resources, and research information resources for the benefit of all parts of the Program. Mr. Kuch participated in the formulation, characterization, and implementation of Branch and Segment projects related to information science and information technology. This participation occurred during a period of substantial growth in the Program's use of various information technologies in the past two years. His collaboration and consultation comprised both assistance on ongoing projects and the development of new projects. For a more detailed description of these projects see the Carcinogenesis Area section of the Annual Report.

He participated in the Information and Resources Segment Advisory Group, which advises Program on the development, approval, and funding of projects in its area of animal, chemical, and information resources. This included planning, organizing, and executing a three-day "Conference on Data Dissemination for Carcinogenesis", which included some fifty information scientists, social scientists, physical scientists, and cancer investigators, and which was charged with advising the Carcinogenesis Program on the opportunities and problems of making large volumes of research data, especially from bioassays, available to those outside the Program.

He was Project Officer on the contract for "Carcinogenesis Bioassay Data System Operational Support". This is a complex support activity involving contract administration files, chemical nomenclature files, animal resource support, provision of information on ongoing bioassays to the International Agency for Research on Cancer, and other functions necessary to the operation of the CBDS. He participated in the CBDS Statistical Group, together with investigators from Carcinogenesis, DCRT, and the Epidemiology Branch, Field Studies Area. This group plans and executes statistical analyses of the Carcinogenesis Bioassay Data System data base. He provided consultation to the Segment Manager, Lung Cancer Segment, on data systems aspects of a research contract with St. Mary's Hospital, Grand Junction Colorado, to study the incidence of lung cancer in smoking and non-smoking uranium miners on the Colorado Plateau. This project has been ongoing for several years, first sponsored by the Public Health Service, then by the NCI. The future of this project may include collaboration with the University of Utah Computing Center, and may be expanded to include CEA analysis, as well as the existing sputum cytology.

The object of this work is to identify pre-cancerous conditions in populations at high risk of lung cancer by means of a cheap and convenient screening process.

He also planned and published the "Collaborative Program Directory" which lists all Carcinogenesis Program contracts, project officers, and other persons of special interest, such as contractor pathologists and toxicologists, and which is in wide use by Program staff and contractors, in response to Program's desire to foster greater collaboration among contractors. He established liaison with, and developed procedures for the use of the National Technical Information Service, Department of Commerce, as a medium for the wider dissemination of research reports of widespread scientific interest, which are too detailed

and lengthy to be appropriate for journal publication. This has reduced considerably the burden of Program in filing, retrieving, copying, and mailing copies of these reports, and has enhanced their public availability.

In the forthcoming fiscal year ADPMS involvement in the Carcinogenesis Program will be limited to occasional consulting on specific projects, including continued participation in the Information and Resources Segment Advisory Group, and continued participation in the data dissemination project, which will involve editing and publishing of Data Dissemination Conference proceedings.

The Viral Oncology Area

Mr. Weiss conducted a detailed study of the Immunology Epidemiology Segment's data management needs and operations at their Bionetics Laboratory facility in order to make recommendations to the Segment Chairman and Bionetics Laboratory regarding systemization and automation of their data activities. Data forms and whole blood, sera, and tissue specimens are gathered from about a dozen research institutions in several areas of the country and submitted to central laboratories where records are maintained and tests are conducted. These data are gathered for studies of cellular immunity in several different diseases. As a result of this study, Mr. Weiss and Mr. Oslik prepared an RFP for the Segment to obtain systems design, computer programming, and data management services under contract. Consultation will continue at a lower level of activity.

OD, DCCP Administrative Management Branch

Until February of this year the ADP Management Section updated its contract summary data on a monthly basis to produce the Contract Commitment Reports for the Division of Cancer Cause and Prevention. In February this extensive operation was turned over to the Research Contracts Office of the Division to be incorporated into their own computerized reporting system.

The ADP Management Section has developed and implemented a management information system for the Division of Cancer Cause and Prevention. This completely automated system generates a series of reports which provide information on computer costs and utilization. One of these reports will be an expenditure report which was originally produced by hand and IBM 2741 on-line terminal. Additionally, a terminal utilization report is being produced to show on-line terminal usage (IBM 2741 Wylbur terminals, for example), for each organization. A report will also be generated to show usage of computer services and equipment by user (programmer or investigator).

Division of Cancer Biology and Diagnosis
Diagnosis Branch

Mr. Weiss has been the principal data processing consultant on the Demonstration Project for the Early Detection of Breast Cancer. Mr. Norman Oslik and Ms.

Caren Parsons have assisted in several areas related to forms and computer systems design. This project is a joint National Cancer Institute - American Cancer Society effort. This project was initiated by the Division of Cancer Biology and Diagnosis and funded under the Cancer Control Program. The major thrust of the start-up effort began this fiscal year.

The overall goals of the project are: to find out if research methods for the detection of breast cancer can be applied to the general population in non-research settings; to determine if a negative thermogram is sufficient, under the conditions of this project, to preclude the need for clinical examination and/or mammography for the detection of breast cancer; to evaluate, insofar as is possible, the sensitivity and specificity of thermography, mammography, and physical examination individually and in combination; to determine if the American Cancer Society can provide a population of women for screening and rescreening on a regularly scheduled basis; to characterize the socioeconomic and medical population of women being screened; to study survival and disease status at follow-up in relation to characteristics of the tumor at time of detection; to find out the extent to which women in the project practice breast self-examination and if not, their stated reasons for non-practice; and to investigate risk factors for breast cancers under the conditions of this project.

At this time it is planned that twenty-seven clinics throughout the United States will each screen five thousand women the first year of the project and an additional five thousand women the second year. Each of these two groups will then be rescreened annually for four additional consecutive years (approximately one million screenings). Each woman will be screened by mammography, thermography, and a physical examination. Cases with findings considered "suspicious" will be rescreened if appropriate, earlier than the annual examination. Cases with findings considered "positive" will be referred to the patients' physicians. Where clinical specimens are taken, data from pathology reports will be obtained. Some seven to ten million documents (some several pages in length) will be generated in this project. Although not all the data generated in the project will be assembled and processed by computer, nevertheless, in terms of the data processing effort involved, this project is quite complex.

Several important elements in the project are being accomplished through the collaborative efforts of the Biometry Branch, the Office of Cancer Control, and the Division of Cancer Biology and Diagnosis, including overall project coordination and direction, statistical consultation, forms design, computer systems planning, contract review, and conference and workshop planning.

In addition to the clinics, a contract has been established to perform all of the data processing work required in this project, including the design, implementation, operation and maintenance of a computer system, the keying of all appropriate data, the preparation and maintenance of procedure manuals, providing training on forms use and procedures, data purification, storage, and

retrieval for analysis, computer facilities and an office for operational planning, document handling and storage.

Mr. Weiss has participated fully in establishing the data management center contract. Because of the extensive amount of data processing and operational work involved in this project, he prepared the Request for Proposal, served as Chairman of the Contract Selection Committee, and presided over the off-site visits by each of the contractors then under consideration. Subsequently, Mr. Weiss became a co-project officer on the data management center contract. A considerable amount of work has been done by this contractor according to Mr. Weiss' overall guidelines and direction. Because of the extensive forms requirements of the project, the considerably large numbers of cases and data processing activities taking place, and the sizeable costs being incurred, careful and continuing review of the data management contractor's activities has been necessary.

The start-up effort can be expected to continue into the next fiscal year, at which time our consulting activities on this project should diminish somewhat. However, as long as the project continues at the present level of complexity and scope, the data management center contract will require continuing review, and the Division of Cancer Biology and Diagnosis and the Office of Cancer Control will require continuing consulting assistance from the Section.

Immunology Branch

The ADPMS is providing data processing consultation to the Immunology Branch of the Division of Cancer Biology and Diagnosis. This Branch is concerned with the chemistry, genetics, biology, and clinical significance of immunity. Studies are concerned with detection and characterization of tumor-specific cell surface antigens and the host's immunological reactions to these antigens.

Mr. Stump is the principal data processing consultant on a study which includes a team of medical staff from the Immunology Branch, DCBD, and a statistical consultant from the Biometry Branch, DCCP. The specific project compares theraputic modalities of patients with malignant melanoma and evaluates the effects of immunotherapy and chemotherapy on patients with Stage II and III malignant melanomas. Data on all patients participating in the study are collected at the staging visit, at all subsequent examinations, and at such time as the patient either dies or is lost to follow-up. Mr. Stump participated in the design of the protocol, the forms, the data base, and the retrieval methodology. In collaboration with the medical staff, Mr. Stump designed and implemented a computer system that edits input data, updates relevant files, schedules patients for their examinations, and produces a variety of reports on selected diagnostic and clinical characteristics.

The batch-type computer system in support of the data collection effort of this study has been in operation since November 1973. An on-line prompting system is being considered as an alternative to the current batch system because errors in data entry have proven to be a problem in system operations.

If implemented, this system would identify errors at the time of data entry and permit the correction of errors before the data are added to the data base. If this recommendation is adopted, it will be implemented during the next fiscal year.

Radiation Branch

In November 1973, the ADPMS began consulting with the Radiation Branch of the Division of Cancer Biology and Diagnosis. This Branch is involved in the development of new cancer treatment techniques using irradiation alone and in combination with other modalities, especially chemotherapy. Clinical investigations currently receiving major emphasis are the malignant lymphomas, chronic leukemias, and bone neoplasms.

Mr. Stump is the primary ADP consultant to Dr. Ralph E. Johnson, Chief of the Branch. Having studied, from a data management point of view, the general operation of the clinical program of the Radiation Branch and having become familiar with the interrelationships between the Branch and the various supporting laboratories and departments, Mr. Stump has recommended the implementation of several ADP techniques. The scope of these recommendations impact upon the record keeping system currently employed by the Branch, the monitoring of patient management, the planning and selection of treatment techniques and the collaborative studies carried out by the Radiation Branch and other program areas which collaborate with the Branch.

During the next six months Mr. Stump will serve as the design analyst for a computer system and will participate in the implementation of his recommendations. During this period he will also conduct a more detailed requirements analysis of the Branch's data management activities, make site visits to several other institutions to identify new methods appropriate for implementation in the Radiation Branch, draw up detailed system specifications, and coordinate all of the data processing aspects of the project. A data processing contract for this work with Mr. Stump serving as co-project officer will be obtained to provide computer programming support.

Office of the Director, NCI

Mr. Kuch provided consultation to the National Cancer Program - Management Information System through membership and participation in the MIS Operations Subcommittee. This group is responsible for coordinating technical aspects of the various subsystems in the MIS, implemented for a variety of organizations within the NCI. These aspects include training documentation, standards, security, procedures, staffing, and subsystem testing.

Significance to Biomedical Research and the Program of the Institute:

The systematic capture, organization, and display of complex and diverse data are of considerable importance in the planning, conduct, and management of the research efforts. As multidisciplinary and collaborative activities increase in scope and complexity, the problems of linking, manipulating, analyzing, and communicating large quantities of data and information become unmanageable without the assistance of computer-related technology.

Proposed Course of the Project:

Consultation and technical support will continue to be provided to the Division's research activities. The level of support is expected to increase in other areas of the Institute.

Honors and Awards

Stump, J. M.: Mr. Stump received a Quality Step Increase this year for continued excellent performance and the development of several techniques of use to the general research program.

Weiss, T.: Co-Project Officer, Division of Cancer Biology and Diagnosis, NCI, Breast Cancer Detection Demonstration Project Central Coordinating and Statistical Center, University City Science Center; Member, Contract Selection Committee, Division of Cancer Biology and Diagnosis, NCI, Central Statistical Group for a Collaborative Study in Early Lung Cancer Screening; Member, Grant Application Review Committee, Division of Cancer Grants, NCI, Comprehensive Cancer Center Grant, University of North Carolina; Member, Contract Review Committee, Division of Heart and Vascular Diseases, NHLI, Central Coordinating Center for Lipid Research Studies, Department of Biostatistics, University of North Carolina; Member, Contract Review Committee, Kidney Transplant Histocompatibility Study, NIAID, Department of Biostatistics, University of North Carolina.

Kuch, T.D.C.: Reviewer, Installation Management Abstracts, Association for Computing Machinery, Special Interest Group on Computer Systems Installation Management; Referee of contributed papers, World Population Society First Annual Meeting; Referee of contributed papers, Association for Computing Machinery, District of Columbia Chapter Annual Symposium; Member, Operations Subcommittee, National Cancer Program - Management Information System Advisory Committee; Member, Chemical Selection Committee, Carcinogenesis Area, DCCP; Member, Information and Resources Segment Advisory Group, Carcinogenesis Area, DCCP; Vice Chairman, Association for Computing Machinery, Special Interest Group on Computers and Society; Meritorious Service Award, Association of Computer Programmers and Analysts.

Publications

Kuch, T.D.C.: Computers in Popular Literature (Series), Computers and Society, 4, No. 2, August 1973.

Kuch, T.D.C.: Collaborative Program Directory, Carcinogenesis Program, 1973.

Kuch, T.D.C., et al: Chapter VII, The Social Security Number as a Standard Universal Identifier, Records, Computers, and the Rights of Citizens, 1973.

Kuch, T.D.C., et al: Chapter VIII, Recommendations Regarding Use of the Social Security Number, Records, Computers, and the Rights of Citizens, 1973.

Kuch, T.D.C., et al: American National Dictionary of Information Processing, 1974.

Kuch, T.D.C.: Computer-Supported Indexing, The Indexer, April 1974.

Kuch, T.D.C.: Review, Friedman, "The Impact of Technology on the Future State of Information Technology Enterprise", Computing Reviews, 14, October 1973.

Kuch, T.D.C.: Review, Kagan and Schear, "The Home Reckoner--A Scenario on the Home Use of Computers, Computing Reviews, 14, October 1973.

Kuch, T.D.C.: Review, Bernstein, "What's in the Cards for Data Entry?", Computing Reviews, 14, October 1973.

Kuch, T.D.C.: Review, McGee, "Summing Up, in the EDP People Problem", Computing Reviews, 1974.

Kuch, T.D.C.: Installation Management Abstracts (Series), Installation Management Review, 2, No. 4, October 1973.

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

Office of the Chief

AICHI CANCER CENTER RESEARCH INSTITUTE (NO1 CP 23213)
KUAKINI HOSPITAL AND HOME (NO1 CP 33216)

Title: Study of Cancer Among Japanese Migrants

Contractor's Project Director: Professor Mitsuo Segi (NO1 CP 23213)
Dr. Grant N. Stemmermann (NO1 CP 33216)

Project Officer (NCI): Mr. William Haenszel

Objectives: The purpose of these contracts is to collect data bearing on the reasons for the differences in cancer incidence between Japanese on the home islands and the Japanese migrant populations in Hawaii and California. The objective is to sort out those aspects of common cancers which may be genetically involved and those which may derive from aspects of the environment or some mixture of the two.

Methods Employed: Standard demographic techniques are employed, basically using a system of diagnosed cases and matched controls. In addition, data have been collected from samples of Japanese households. The matched case-control technique is particularly useful in this kind of study. Pathology protocols are being completed for cases in the study series for several sites (stomach, colon, rectum) and considerable emphasis is being placed on the correlation of the epidemiological and pathology findings. In preparation for later case-control studies of prostate cancer, latent prostate cancer is being studied in 5 groups of patients.

Major Findings: Study of 179 Japanese patients with bowel cancer and 357 hospital controls in Hawaii revealed excess risks for persons who regularly ate Western-style meals only. The similarity in findings for Issei and Nisei sharply contrasts with results previously reported for stomach cancer in Issei and Nisei, where the reduced risk among persons regularly eating Western-style meals only was limited to the second-generation Nisei. Bowel cancer was associated with consumption of specific foods. Bowel cancer patients ate meats, legumes, and starches more frequently, with the case-control disparity being less pronounced for individuals consuming starches than for those eating meat and legumes. Beef and string beans were the major contributors to the meat and legume effects. There seemed to be separate effects for beef, string beans, and starches, and the case-control contrasts were strengthened when various combinations of foods were considered. These results may narrow the area of search for etiologic factors and thus assist in planning future research on the complex sequence of events leading to bowel cancer.

The cancer risks for Japanese migrants differ from those observed in Japan; the migrant risks are higher for lung, colon, rectum, prostate and lower for stomach while little change has been observed for breast among post-menopausal women. The case-control studies show an excess consumption of salted and dried fish and pickled "salted" vegetables some of which are rich in secondary amines, by stomach cancer patients. Since nitrites and secondary amines can be synthesized in vivo into nitrosamines, potent carcinogens, a possible etiology is suggested for further study.

Latent prostate cancer is almost as common in Japanese as in Americans though Japanese have little clinical cancer of this site. A possible resolution of this anomaly has been contributed by Dr. Akazaki, who has described and defined two types of latent carcinoma - proliferative and non-proliferative. The gradient in prevalence of the proliferative type lesion in his autopsy studies of specimens from Japan and Hawaii Japanese corresponds closely to the gradient in incidence of clinical prostatic carcinoma in the two populations. The important feature of the Hawaii Japanese males is the greater amount of proliferation and not their unremarkable overall prevalence of occult lesions, which suggests the presence of some promoting factor in the migrant male population.

Significance to NCI Program and Biomedical Research: If the studies in these migrant populations elucidate the environmental factors involved in the incidence of certain forms of cancer, and indicate those that are predominately environmental and those which are predominantly genetic, then it may be possible to apply these findings to the United States population for purposes of cancer prevention and cancer control.

Proposed Course: These two contracts are expected to continue for several years because of the long range nature of these studies. The male cohort of Japanese being followed at Kuakini may be expanded to include sisters, wives and daughters.

Date Contract Initiated: February 1972 (NO1 CP 23213)
June 1971 (NO1 CP 33216)

Current Annual Level: $163,389 (NO1 CP 23213)
$529,303 (NO1 CP 33216)

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

Office of the Chief

AMERICAN CANCER SOCIETY (NO1 CP 43213)

Title: Study of Smoking and Mortality Among Veterans

Contractor's Project Director: Dr. E. Cuyler Hammond

Project Officer (NCI): Mr. William Haenszel

Objectives: This project is for the final collection of data for the study of smoking and mortality among U.S. Veterans started by Dr. Harold Dorn in 1954. The original cohort was comprised of 293,658 veterans who held U.S. Government Life Insurance policies. A great amount of data has been collected on these people, but the information from 32,000 death certificates since 1962 still remains to be obtained and coded.

Methods Employed: Dr. Hammond of the American Cancer Society will obtain and code these 32,000 death certificates in conjunction with the current mortality follow-up of his smoking study cohort of over one million persons. He estimates that 25,000 of these records can be obtained routinely, but that the rest will require a search in various health departments by ACS field personnel.

Major Findings: This is a new contract and no results are available yet.

Significance to NCI Program and Biomedical Research: This contract will allow completion of a major long term study on smoking and health, and will result in a series of important papers on this cohort, the ACS cohort and a cohort of English physicians.

Proposed Course of Project: This study is expected to be completed within 3 years.

Date Contract Initiated: July 1, 1973

Current Annual Level: $100,877

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

OFFICE OF THE CHIEF

CALIFORNIA STATE DEPARTMENT OF HEALTH (NO1-CP-33353)
CONNECTICUT STATE DEPARTMENT OF HEALTH (NO1-CP-43203)
MICHIGAN CANCER FOUNDATION (NO1-CP-43264)
UNIVERSITY OF IOWA (NO1-CP-43200)
UNIVERSITY OF NEW MEXICO (NO1-CP-23235)
FRED HUTCHINSON CANCER RESEARCH CENTER (Seattle) (NO1-CP-33246)
UNIVERSITY OF UTAH (NO1-CP-33354)
RESEARCH CORPORATION OF THE UNIVERSITY OF HAWAII (NO1-CP-33261)
LOUISIANA SOCIAL AND REHABILITATION SERVICES ADMINISTRATION (NO1-CP-43262)

Title: Surveillance, Epidemiology, and End Results (SEER) Program

Project Officer (NCI): Sidney J. Cutler, Sc.D.

Objectives: To obtain and analyze data on morbidity, mortality, and survival from population-based registries, to identify areas for epidemiologic investigations, and to initiate preliminary investigations needed to develop epidemiological study protocols.

Methods Employed: The National Cancer Institute is sponsoring a collaborative program for Cancer Surveillance, Epidemiology, and End Results Reporting (SEER Program). Participants in this program are all population-based registries, covering entire states or geographically specified areas, usually Standard Metropolitan Statistical Areas. The Biometry Branch has professional and technical responsibility for supervising these contracts. Basic information is collected on all patients treated for cancer in the area, area residents treated outside the area, as well as anyone dying in the area where cancer is mentioned anywhere on the death certificate - or area residents dying outside the area, where cancer is mentioned anywhere on the death certificate. Periodic follow-up information is obtained on the vital status of all those in the registries or from a complete geographic subsample. Data is submitted annually to NCI on uniformly formated computer tapes. These data are available for analysis, as well as for providing an extensive resource for special studies.

Core epidemiological staff are present in most of the SEER programs. These staff members will review the morbidity data to identify epidemiological research leads that should be investigated. This staff will develop the study protocol and field-test the study schedules. At this point, the program would request separate funding from NCI, either through contract or grant mechanism, or seek financial assistance from other sources.

Significance to NCI Program and Biomedical Research: Information on incidence, survival, and mortality from a representative cross-section of the U.S. population is required on a continuing basis so that the nature and magnitude of the cancer problem including changes over time can be determined. Through close scrutiny of variation in cancer incidence and survival with respect to geographic and demographic characteristics of the population and differential changes over time, specific etiologic hypotheses will emerge which, when tested via special study mechanism, should then lead to the identification of controllable risk factors. Such research is an integral part of the ultimate goal of the national program: the reduction in the occurrence of and mortality due to cancer. The SEER Program would also serve in the evaluation of cancer control programs conducted in the areas covered by the registries. They would also provide the mechanism for identifying patients who had suspicious or positive findings on screening tests but were either not followed for any extended period of time or were lost to follow-up, and who were eventually diagnosed with a malignant neoplasm.

Major Findings: The SEER Program is a new venture. Data on morbidity from all areas will start being received by NCI for the year 1973 by the end of 1974. It will require a number of years of checking and evaluation to insure that all areas are submitting accurate, complete, and comparable data. A continuing series of reports will be generated once the above has been completed.

Proposed Course of Projects: Emphasis in the next few years will be toward: 1) insuring that each program is accessing all of the cases with a reportable disease in the specified geographic area covered by the registry; 2) training of personnel in the codes and procedures to insure accurate, complete and comparable data; 3) developing follow-up procedures for patients who are in the End Results evaluation portion to insure a high percentage of successful follow-up.

	Year Started	FY-74 Obligation
California State Department of Health (NO1-CP-33353)	1962	$785,000
Connecticut State Department of Health (NO1-CP-43203)	1963	276,041
Michigan Cancer Foundation (NO1-CP-43264)	1973	361,314
University of Iowa (NO1-CP-43200)	1973	241,493
University of New Mexico (NO1-CP-23235)	1973	369,845
Fred Hutchinson Cancer Research Center (Seattle), (NO1-CP-33246)	1973	350,000*
University of Utah (NO1-CP-33354)	1973	150,000*
Research Corporation of the University of Hawaii, (NO1-CP-33261)	1971	93,000
Louisiana Social and Rehabilitation Services Administration (NO1-CP-43262)	1973	151,979

*Estimates of 1974 obligations

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
FISCAL YEAR 1974

Office of the Chief

HEALTH INSURANCE PLAN OF GREATER NEW YORK (N01 CP 43278)

Title: Evaluation of Periodic Breast Cancer Screening by Mammography

Contractor's Project Director: Dr. Raymond Fink

Project Officer (NCI): Dr. Sidney Cutler

Objectives: A. To determine whether a simple screening technique (x-ray mammography) can diagnose breast cancer early.

B. To determine if the very early diagnosis of breast cancer is really useful in improving long-term survival.

C. To investigate through a prospective study of the women screened, the relationship of a wide range of parameters to the development of breast cancer.

Methods Employed: This is a combination statistical-epidemiological-medical study in which a study population receiving mammography at annual intervals and a control group of 30,000 women each are being routinely followed for breast cancer experience through internal Health Insurance Plan files and through review of death certificates. Details of the techniques are given in a paper describing the methodology and initial findings of this study entitled, "Evaluation of Periodic Breast Cancer Screening with Mammography" by Sam Shapiro, Philip Strax, and Louis Venet, JAMA 195: 731-738, 1966.

During one reexamination cycle 40 cc of blood was taken from women in the study population and stored.

Major Findings: Findings to date strongly suggest the usefulness of annual screening which includes mammography. Over the short run period of 6 years of follow-up, the study group of women have about a one-third less mortality from breast cancer than those in the control group. The differential between control and study cases in fatality from breast cancer is almost entirely due to the exceptionally low rate among the cases detected through screening. Both the clinical examination and mammography contributed to this favorable situation but mammography, with a fatality rate of only 2% among cases detected in the absence of positive clinical findings during screening, was especially important.

In examining various risk factors for the women who appeared for an initial screening the following women were found to have a 20-30% increase in risk of breast cancer: higher educated women, Jewish women, and unmarried women. Increasing age at first pregnancy, low gravidity, and menarche at ages under 15 are associated with a 50-100% increase in risk.

Significance to NCI Program & Bio-Medical Research: Early diagnostic procedures may be of use in the potential cure of cancer patients. It appears that mammography is such a technique, and is being extended to other populations.

Proposed Course of Project: The screening part of the study is complete and the emphasis from now on will be on follow-up. The women will be followed for as long a time period as necessary to demonstrate whether the technique is truly useful as an early diagnostic device with long term impact on mortality from breast cancer. The duration of the study is contingent upon the answers to the questions implied by the objectives.

Supplemental lines of inquiry may be suggested by the Breast Cancer Task Force. Proposals for use of the stored blood plasma are being developed.

Date Current Contract Initiated: November 1, 1962

Current Annual Level: $65,376

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

Office of the Chief

ISRAEL CENTER FOR REGISTRATION OF CANCER AND ALLIED DISEASES (NO1-CP-33351)

Title: Cancer Incidence Study and Registry Assessment

Contractor's Project Director: Dr. Ruth Steinitz

Project Officer (NCI): Dr. Sidney J. Cutler

Objectives: Approved as part of a three year project, the first two years are devoted to collection of cancer incidence data so that a report in the third year may analyze such cancer incidence for the 12 years 1960-71 with complete coverage of the population of Israel. As the study progresses, additional reports are produced starting with assessment of the registry's collecting system and in the third year assessment of the data processing system. Therefore, part of the objectives are to indicate what changes are required to produce useful epidemiologic data more quickly.

Major Findings: During the first two years changes in personnel practice have been instituted and some changes in technology. The collection and processing of data are on schedule. However, the recent warfare may have delayed the release of official census data so that only a preliminary analysis can be produced in the third year.

Significance to Biomedical Research and the Program of the Institute: Because of the large number of ethnic and immigrant groups, Israeli cancer data have unique epidemiologic potential and fit well into the Branch's collaborative study program involving changes in incidence and mortality after migration. Of major importance is the fact that specific differences in cancer incidence between population subgroups are reported from Israel which far exceed those found between different population groups in the U. S. The population-based cancer registry can also be integrated in the long run with the "SEER" Program (Collaborative Program for Cancer Surveillance, Epidemiology, and End Results Reporting) based upon domestic cancer data.

Proposed Course: This contract was to be renewed annually until the report due at the end of three years had been received, after which a critical review was planned to see whether further support would be justified in establishing specific epidemiologic studies. It may now be necessary to fund at least part of the fourth year to obtain a useful incidence study. As indicated above, the unknown factor is the date of release of detailed census information.

Date Contract Initiated: June 28, 1972

Current Contract Level: $35,030

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

Office of the Chief

JOHNS HOPKINS UNIVERSITY (NO1 CP 12422)

Title: Epidemiological Study of Rare Sites of Cancer

Contractor's Project Director: Dr. Abraham M. Lilienfeld

Project Officer (NCI): Mr. William Haenszel

Objectives: To elucidate the causal factors in the development of selected rare cancers in patient groups which are large enough to make the findings valid and reliable. The rare sites to be studied will be liver, gallbladder, islet cell tumors of the pancreas, renal pelvis, nasopharynx, adrenal gland, testis, penis, scrotum, vagina, vulva, and male breast.

Methods Employed: The investigation is in the form of a case-control study. The appropriate cases and matched controls are identified, interviews are obtained and medical records checked. A total of five areas are included, two of which are also part of the Third National Cancer Survey: Buffalo, Detroit, Miami, Minneapolis-St. Paul, and New York City.

Major Findings: The major finding of this study so far is that it takes a great deal of time and effort to locate new cases with these rare cancers as well as appropriate controls. It is also difficult to find the new cases soon enough after diagnosis to interview them while still in the hospital. The original plan to work through the case finding systems established by the field offices of the Third National Cancer Survey did not prove feasible, and therefore, other arrangements were made in other metropolitan areas. Less than 100 cases of any one rare cancer site have been found since the inception of the study in June 1971 and adequate numbers of controls have not been obtained.

Significance to NCI Program and Biomedical Research: This study was designed to fill the information gap regarding the causative and related factors associated with the rare types of cancer. New knowledge from such a study could be useful in the control of the more common types of cancer as well.

Proposed Course of Project: Because of both technical and administrative problems, the contract is being phased out during a terminal year period which provides for orderly conclusion of the field work and adequate time for analysis of the data in hand.

Date Contract Initiated: June 28, 1971.

Current Annual Level: None

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

Office of the Chief

LOUISIANA STATE UNIVERSITY MEDICAL CENTER (NO1-CP-43281)

Title: Premalignant Lesions in the Large Intestine

Contractor's Project Director: Professor Jack P. Strong

Project Officer (NCI): Dr. James L. Murray

Objective: To obtain information on the frequency and extent of various kinds of pathologic changes, especially polyps and benign tumors, in the intestinal tracts of black and white Americans. This information will be compared with that from several other populations of epidemiologic interest.

Methods Employed: Tumors are identified by a careful protocol study of bowel specimens obtained at autopsy.

Major Findings: In the first two years supported by this contract they have collected approximately 530 complete specimens of large intestines at autopsy from individuals in New Orleans. These specimens were collected in order to make comparisions among age, sex, and race subgroups with similar subgroups in other geographic locations. The specific goal has been to collect enough cases to make meaningful comparisons among subgroups of a total of 1500 cases collected and analyzed by Dr. Pelayo Correa in Cali, Colombia. Lesions in the large intestine have been identified, enumerated, and classified in an objective standardized fashion similar to that used in Cali, Colombia. They have conducted workshop conferences on measurement error and reproducibility. These data have been providing useful information on evaluating the lesions. From the 462 cases studied so far there were 174 with adenomas and 7 with carcinomas. Eighty-five cases with single adenomas and 89 specimens with multiple adenomas were found. The number of individuals 45 years of age and older was 384. Of these, 166 or 43% contained adenomas.

Significance to NCI Program and Biomedical Research: It is hoped to identify those epithelial lesions which are epidemiologically related to colon cancer. This will not only provide clues on the pathogenesis but identify individuals at high risk for colon cancer and provide a signal of exposure closer in time to the contact of patient and environmental factors.

Proposed Course: This contract will continue 2 more years for data collection and analysis.

Date Contract Initiated: June 1, 1971.

Current Annual Level: $33,036

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

Office of the Chief

NEW YORK MEDICAL COLLEGE (NO1 CP 33321)

Title: Study on Breast Lesions in Relation to Changing Incidence Rates in Breast Cancer

Contractor's Project Director: Maurice M. Black, M.D.

Project Officer (NCI): Dr. Benjamin F. Hankey

Objective: To examine the increasing trend in the incidence of breast cancer over the past two and one half decades in terms of possible changes in diagnostic criteria. Survival data is also being collected. It should be noted that during this period breast cancer mortality has remained relatively constant. This is one of two contracts associated with this study. The other is with the SASKATCHEWAN CANCER COMMISSION (NO1 CP 23276).

Methods Employed: Samples of patients with benign lesions and samples of patients with malignant lesions are identified in the two cancer clinics in the province of Saskatchewan, Canada. The slides for these patients are read by a pathologist and the differences in diagnosis between what was originally made and what was made in our study are examined.

Major Findings; It does not appear that changes in diagnostic criteria explain the increasing trend for breast cancer in Saskatchewan, however, the histology data indicate that there may be changes in the characteristics of the disease over the period of study (1946-1968).

Significance to NCI Program and Biomedical Research: This project fits well into the series of ongoing attempts to understand apparent trends in the incidence of specific cancers and also provides survival information on various histologic prognostic factors.

Proposed Course: More than half of the data has been collected and analyzed. Data will continue to be collected over the remaining period of the contract and analyzed in accord with contract objectives.

Date Contract Initiated: June 24, 1971.

Current Annual Level: $47,900

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

Office of the Chief

NORWEGIAN PUBLIC HEALTH SERVICE (NO1 CP 43247)
UNIVERSITY OF MINNESOTA (NO1 CP 33325)

Title: Incidence, Prevalence and Mortality from Cancer in Selected Migrant Populations (Norwegian)

Contractor's Project Director: Dr. Einar Pedersen (NO1 CP 43247)
Dr. Leonard Schuman (NO1 CP 33325)

Project Officer (NCI): Mr. William Haenszel

Objectives: To determine the reasons for difference in cancer incidence and mortality in sedente populations and migrant populations of the same ethnic and genetic composition.

Methods Employed: Standard epidemiologic-demographic methods including prospective observations on defined cohorts and case-control studies.

Major Findings: A case-control study of cancer of the stomach and large bowel in Norwegians in Norway and Minnesota has been completed by Dr. E. Bjelke. The pattern of differences between these two population groups is similar to that found in the Japanese in Japan and Hawaii.

The introduction of a personnel identification number has made record linkage a simple operation, made a large number of studies possible, and facilitated keeping track of cohorts used in the migrant studies. The personnel identification number system is being used to link current study records with data from the 1960 Norwegian census to better identify the characteristics of various population groups. This enabled a study of nickel workers to be accomplished at a much lower cost than would be possible in the United States of America.

The Norwegian Registry also provides one of the means for doing ad hoc international studies. For example, there is interest in studying the effect of differences in incidence rates for such sites as breast and large bowel on the stage at diagnosis and the survival rates. These studies are done more effectively using different countries for study populations.

Significance to NCI Program and Biomedical Research: This is one of a group of migrant studies to determine those elements in the incidence and mortality from cancer which may be related to environment and those which may be related to genetic factors. Isolation of environmental elements may be useful in cancer prevention within the United States.

Proposed Course of Project: This project in Norway and Minnesota will continue for several more years. The basic migrant study will continue and analysis of the data on respiratory cancers and gastrointestinal cancers will be accelerated. Plans are being made to extend the survival analysis of end results data collected by the Registry during the period 1953-67. New studies may also be started on breast cancer, thyroid cancer and Hodgkin's disease.

The University of Minnesota has defined a cohort of approximately 20,000 policyholders in an insurance company that has a high proportion of persons of Norwegian descent and will monitor the group prospectively for mortality data. The contractor has also carried out case-control interviews for gastrointestinal cancer among persons of Norwegian descent in Minnesota and is prepared to extend field work to other sites at a later date. All phases of the cohort and case-control studies in Minnesota are closely coordinated with the ongoing work in Norway. One investigator from the Cancer Registry of Norway frequently visits the University of Minnesota to assure close liaison.

Date Contract Initiated: March 1964 (NO1 CP 43247)
June 1966 (NO1 CP 33325)

Current Annual Level: $65,000
$15,547

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

Office of the Chief

RESEARCH CORPORATION OF UNIVERSITY OF HAWAII (NO1 CP 33261)

Title: Demographic Cancer Research and Training Program in Hawaii

Contractor's Project Director: Dr. Richard K. C. Lee

Project Officer (NCI): Mr. William Haenszel

Objectives: The objective of this project is to plan and develop a Demographic Cancer Research and Training Program in Hawaii. This Program will become an integral part of a new comprehensive cancer research center in Hawaii.

Methods Employed: Standard methods of planning, programming and budgeting, as well as personnel recruitment and research management are employed.

Major Findings: As a direct result of the planning efforts under this contract, the Research Corporation has been successful in securing a Cancer Center Support Grant for the operation of a new cancer research center in Hawaii which will incorporate and coordinate all the major medical interests in cancer in the State of Hawaii. The Demographic Cancer Research and Training Program will continue to be closely related to the new center program and they will be mutually supportive. The Hawaii Tumor Registry is being supported to improve its operation and data base. It will eventually have a rapid incidence reporting system, and will become a vital and integral part of the new cancer center program. The Data and Computation Program at the University has developed a computerized population-based family file for all citizens of Hawaii. It contains demographic data and vital statistics on the population for the past 30 years, and these records are being linked via computer with new vital statistics data and Tumor Registry data, and have also been linked with data from the Hawaii Cooperative Chemotherapy Program. The Training Program included M.P.H., Ph.D. and residency programs which are available to research associates working on other parts of the Demography Program in Hawaii. The Japanese migrant studies are continuing on cancers of the esophagus, lung and gallbladder. Information on new cases and controls is being obtained by personal interview from seven of the major hospitals in Hawaii. Also, the interviewing staff continues to assist with the examination of certain patients and cohort members of the Japan-Hawaii Cancer Study at Kuakini Hospital.

Significance to NCI Program and Biomedical Research: This Demographic Cancer Research and Training Program has formed the basis for a comprehensive cancer research effort in Hawaii. It is the foundation of the new cancer center and

plays a key role in upgrading the Hawaii Tumor Registry to participate in the SEER Program.

Proposed Course of the Project: This project will continue for several years in close cooperation with the cancer center grant.

Date Contract Initiated: June 15, 1971.

Current Annual Level: $400,000

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

Office of the Chief

SASKATCHEWAN CANCER COMMISSION (NO1 CP 23276)

Title: Study on Breast Lesions in Relation to Changing Incidence Rates in Breast Cancer

Contractor's Project Director: Dr. T.H.C. Barclay

Project Officer (NCI): Dr. Benjamin F. Hankey

Objective: To examine the increasing trend in the incidence of breast cancer over the past two and one half decades in terms of possible changes in diagnostic criteria. Survival data is also being collected. It should be noted that during this period breast cancer mortality has remained relatively constant. This is one of two contracts associated with this study. The other is with NEW YORK MEDICAL COLLEGE (NO1 CP 33321).

Methods Employed: Samples of patients with benign lesions and samples of patients with malignant lesions are identified in the two cancer clinics in the province of Saskatchewan, Canada. The slides for these patients are read by a pathologist and the differences in diagnosis between what was originally made and what was made in our study are examined.

Major Findings: It does not appear that changes in diagnostic criteria explain the increasing trend for breast cancer in Saskatchewan, however, the histology data indicate that there may be changes in the characteristics of the disease over the period of study (1949-1968).

Significance to NCI Program and Biomedical Research: This project fits well into the series of ongoing attempts to understand apparent trends in the incidence of specific cancers and also provides survival information on various histologic prognostic factors.

Proposed Course: More than half of the data has been collected and analyzed. Data will continue to be collected over the remaining period of the contract and analyzed in accord with contract objectives.

Date Contract Initiated: June 21, 1972

Current Annual Level: $14,270

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

Office of the Chief

UNIVERSIDAD DEL VALLE -- FUNDACION PARA LA EDUCACION SUPERIOR (NO1-CP-33286)

Title: Epidemiology-pathology studies of cancer in Colombia

Contractor's Project Director: Dr. Carlos Cuello

Project Officer (NCI): Mr. William Haenszel

Objectives: To determine site-specific cancer incidence in Cali and other cities in Colombia and to correlate the morbidity data with pathology findings.

Methods Employed: The cancer morbidity surveys in Cali will be continued with emphasis on variation by socioeconomic class and place of birth. Internal migration within Colombia is of such magnitude that two-thirds of the residents of Cali were born elsewhere. Cancer morbidity surveys on a limited scale are being undertaken in Cartegena and Medellin, cities thought to have greatly different site-specific incidence, and in the Department of Nariño.

Major Findings: Studies of autopsy materials and surgical specimens are being conducted in Cali to define criteria which differentiate high-risk and low-risk populations. In Cali an organ bank of tissues from persons dying from accidents and acute diseases is being utilized for study of occult tumors and malignant precursors with emphasis on stomach, colon and cervix. The organ bank findings also emphasize the distribution by place of birth and are used to complement the incidence data from the morbidity surveys. Similar pathologic material is being collected in New Orleans and Hawaii.

The Cancer Registry in Cali is analyzing its data from the past ten years and preparing them for publication in the next edition of Cancer Incidence in Five Continents. The work on colon cancer is being conducted primarily in Cali and Medellin. Special studies are continuing on the precise localization of the colon cancer lesions and their histologic characteristics. A comprehensive epidemiologic study of stomach cancer is being conducted, primarily in the Department of Nariño. The problem may be related to nitrates in the water supplies. Incidence data will continue to be collected and analyzed to further identify high risk areas and subgroups of people. Studies of lymphoreticular tumors are continuing and are directed toward elucidation of environmental and host factors. Some cooperative efforts with the International Union Against Cancer (UICC) may be developed in the future. The collaborative study of thyroid cancer with investigators in Hawaii has been completed. Occult carcinomas were found in 5.6% of the autopsy samples which is similar to that found in the United States. A similar

collaborative study on prostate cancer is being conducted with investigators in Japan to assess the frequency of occult cancerous lesions in the male population. A histologic study of cervical cancer is underway in Cali and a mixed cell type tumor is often seen which does not fit into the usual Reagan classification system.

Significance to NCI Program and Biomedical Research: This contract is producing significant data for comparative studies in the United States and elsewhere, with emphasis on those areas providing the greatest contrasts in risks for specific cancer sites.

Proposed Course of Project: The various aspects of this project will continue for at least three years. Emphasis will be placed on clinical and experimental studies of the genesis of intestinal metaplasia and carcinoma of the stomach. New collaborative research efforts will begin with investigators in Brazil, Costa Rica and Venezuela.

Date Contract Initiated: June 29, 1966.

Current Annual Level: $98,684

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

Office of the Chief

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY (NO1 CP 12427)

Title: Comparative Fecal Flora Studies

Contractor's Project Director: Dr. W. E. C. Moore

Project Officer (NCI): Dr. Sidney J. Silverman

Objectives: To compare the intestinal microflora of Japanese migrants to Hawaii who have intestinal polyps with those without such lesions. To determine the effect of diet on the intestinal flora and to study the neutral and acid steroids in the feces of the individuals under study.

Major Findings: Bacteriological identification has been completed of the isolates from normal Japanese-Hawaiians and study is in progress of polyp patients from this population and of Japanese living in Japan. Of the 1147 isolations obtained from the normal population study, 113 different types were detected. The isolates represent over 90% of the microbial population in these samples. Correlation of the microbiological observations and the steroid analyses with the diet and clinical histories obtained from the subjects is still to be done.

Significant differences were observed in Japanese-Hawaiians with a history of intestinal polyps and normal subjects in the metabolism of cholesterol and the plant steroids, sitosterol and campesterol. Polyp patients showed little or no conversion of neutral steroids by the intestinal bacteria, while the expected conversion to coprostanol and coprostanone was observed in the feces of the controls. Conversion or the absence of conversion is a stable characteristic of an individual. The feces of polyp patients also contained less total neutral steroids than did the controls. Study of 31 North American caucasians showed that a minority of this group also failed to convert cholesterol. This may indicate a difference in the degree of risk between convertors and nonconvertors. No significant difference in the degradation of bile acids was detected when the Japanese polyp patients, normal subjects, and North American caucasians were compared. Only lithocholic acid was significantly lower in the polyp population.

Significance to Biomedical Research and the Program of the Institute: This project is part of the greater epidemiological study that will correlate laboratory, clinical, and demographic data to elucidate the factors causing intestinal carcinomas. It will help to answer the questions concerning the interrelationships between diet, intestinal microflora, bile acid components, and tumor formation. The project provides cultures for metabolic studies at the Frederick Cancer Research Center.

Proposed Course: The identification of microorganisms and the analyses of data will be completed during the coming year. The contract will be transferred to the Carcinogenesis Program.

Date Contract Initiated: June 30, 1971.

Current Annual Level: $224,000

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

Office of the Chief

YALE UNIVERSITY SCHOOL OF MEDICINE (NO1 CP 33235)

Title: Development of a Connecticut Cancer Epidemiology Program

Contractor's Project Director: Dr. Robert McCollum

Project Officer (NCI): Mr. William Haenszel

Objective: To develop a cancer epidemiology program with the primary purpose of studying and utilizing the data in the Connecticut Tumor Registry (CTR). Because of personnel restrictions imposed on the CTR by the State of Connecticut, the CTR staff is not in a position to analyze the incidence and mortality data nor to use the information in epidemiologic studies of cancer in Connecticut.

Methods Employed: Epidemiologists and statisticians will be recruited to develop a staff for the project which will have direct access to the data in the registry. The unit will not only conduct its own research and educational activities utilizing the CTR but will also actively encourage others to use and expand the resources of the registry.

Major Findings: A senior epidemiologist has recently been recruited to direct the new research unit, and a research program is being developed. Supporting staff are being recruited gradually to achieve controlled growth of a well-organized program. Arrangements are being made to assure close working relationships with the Registry and with the hospitals and practicing physicians in the state to facilitate future studies.

Significance to NCI Program and Biomedical Research: The Connecticut Tumor Registry has been for years the cornerstone of the information reporting system which supports a major part of the Field Studies and Statistics Program. It provides incidence data, End Results data, and related information for epidemiologic studies, both national and international. It is important that all this information be used to the maximum possible extent in order to advance our knowledge of the natural history of cancers and to capitalize on our long term investment in the CTR.

Proposed Course of Project: This project is expected to gradually develop into an important epidemiologic research center in cooperation with the Connecticut Tumor Registry.

Date Contract Initiated: February 1, 1973.

Current Annual Level: $55,000

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

ADP Management Section

MARYLAND NATIONAL OPTIMATION SERVICES, INC. (NO1-CP-12050)

Title: Data Conversion for the Third National Cancer Survey

Contractor's Project Director: Daniel Wallingsford

Project Officer (NCI): Theodore Weiss

Objectives: Convert the data on approximately seven hundred and fifty thousand Third National Cancer Survey documents to magnetic tape.

Major Findings: Data are being converted by the optical character recognition method (OCR). The contractor is using a system of procedures and computer programs specifically designed for him by the Institute. The system includes extensive security control over documents and information, a pre-edit of completed Survey documents, and manual and computer edit checks of the data transcription process.

Significance to Biomedical Research and the Program of the Institute: See Third National Cancer Survey Project Report, Demography Section, Biometry Branch, NCI.

Proposed Course: Contract will terminate after the patient interview booklet data are captured (approximately September 30, 1974).

Date Contract Initiated: October 19, 1970

Current Contract Level: $277,746

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

ADP Management Section

WOLF RESEARCH AND DEVELOPMENT CORP. (N01 CP 33254)

Title: Computer Programming Services for the Third National Cancer Survey

Contractor's Project Director: Edwin Lisiecki

Project Officer (NCI): James E. Larson

Objectives: This contract provides biomedical computing software support to the Third National Cancer Survey project.

Major Findings: Wolf is providing systems analysis and computer programming support to the Special Cancer Survey Section for the data processing and preliminary analysis phases of the Third National Cancer Survey. Specific activities include:

- the operation, maintenance and orderly close down of the Survey's data processing system;
- the modification of an existing sub system to produce incidence data for all three survey years (1969, 1970, 1971) along with a final detailed edit of all cases on file to facilitate the resolution of data discrepancies prior to the termination of field office activities;
- the development of numerous computer programs to support the data purification and analysis phases of the Special Skin Cancer Study;
- the development of a sub system to capture, edit, and analyze Patient Interview data;
- the production of reports for the extended analysis of the Survey's "payments to hospitals" data;
- the detailed geographic coding of the data base;
- the generation of all statistical tables for an Advanced Three Year Report and an NCI Monograph; and
- technical support for numerous special studies.

Significance to Biomedical Research and the Program of the Institute: See Projects Reports, Demography Section, Biometry Branch, NCI.

Proposed Course: The support represented by this contract will continue through September 30, 1974.

Date Contract Initiated: February 11, 1973

Current Contract Level: $213,677

CONTRACT NARRATIVE
BIOMETRY BRANCH, DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

Clinical and Diagnostic Trials Section

WOLF RESEARCH AND DEVELOPMENT CORPORATION (NO1CP-33259)

Title: Computer Services

Contractor's Project Director: Don Corle

Project Officer (NCI): Dr. David P. Byar

Objectives: This program provides systems design and computer programming support for projects in the Clinical and Diagnostic Trials Section.

Major Findings: During the past year the contractor has maintained a Veterans Administration Cooperative Urological Research Group (VACURG) computer system comprised of approximately 40 computer programs which edits new data, updates master files, and produces a semi-annual report containing numerous tables and other data displays. This document consists of some 120-150 pages of extensive tabulations of data. The results are derived from complex statistical analyses, sophisticated data handling and analytical techniques, and data graphically plotted by digital computer. Since important clinical decisions are based on this report, up-to-date data must be presented as quickly as possible. Much of the computer output, therefore, must be in a form suitable for direct photographic copying. Before the report can be prepared, the data bases of five major clinical trials are updated with follow-up information received since the last report, and then submitted for a thorough computer editing.

Wolf personnel have written numerous computer programs for special studies related to the VACURG study and for other selected studies in which the Section participates. This often involves developing computer programs to facilitate the study of properties of medical and demographic data utilizing complex analytical techniques and decision rules.

In addition Wolf personnel have designed a system for recording the results of some twelve laboratory tests which are done on all patients in Prostate Studies II and III every six months. The system provides for entering this large volume of data directly into the computer system at our reference biochemistry laboratory in Minneapolis on a daily basis as the work is completed. Computer programs have been written to perform the many repetitious and complex calculations needed to convert the raw laboratory data into meaningful lab values. The complete system of editing and updating for the main file has also been designed and the results of the tests are sent periodically to the Section office for a statistical analysis in relationship to the patient's clinical records.

Proposed Course: During fiscal year 1973 this contract was extended for a further year and is currently scheduled to be in operation through the end of February 1975.

Date Contract Initiated: February 28, 1972

Current Contract Level: $90,000

CONTRACT NARRATIVE
BIOMETRY BRANCH, OADFS&S
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

Demography Section

UNIVERSITY OF ALABAMA MEDICAL CENTER (Birmingham) (NO1-CP-90049)
COLORADO DEPARTMENT OF PUBLIC HEALTH (Denver) (NO1-CP-90050)
COMMONWEALTH OF PUERTO RICO, DEPARTMENT OF PUBLIC HEALTH (San Juan) (NO1-CP-90072)
MEDICAL ASSOCIATION OF GEORGIA (Atlanta) (NO1-CP-90047)
UNIVERSITY OF MINNESOTA, SCHOOL OF PUBLIC HEALTH (Minneapolis) (NO1-CP-90045)
UNIVERSITY OF PITTSBURGH (NO1-CP-90043)
UNIVERSITY OF TEXAS, SOUTHWESTERN MEDICAL (NO1-CP-90040)
MAYO FOUNDATION (Minnesota) (NO1-CP-02057)

Title: Third National Cancer Survey

Contractor's Project Director: See table on following page

Project Officer (NCI): Sidney J. Cutler, Sc.D.

Objectives: Procurement and initial processing of data for the Third National Cancer Survey

Major Findings: An advanced report of cancer incidence 1969-1971 in all areas combined was released in February. Reports for each individual area are being prepared for release in April. An NCI monograph containing incidence data by site and type of cancer for all areas combined and for each area separately is scheduled for release in June. Reports containing information on all cancer patients seen by each hospital participating in the survey were prepared and distributed to each hospital for use in their own hospital registry.

Significance to Biomedical Research and the Program of the Institute: See Individual Project Report NCI-4268.

Proposed Course: All data collection has been completed. Field offices are operating on a core staff of one to two persons. Three field offices have been absorbed into ongoing registry programs which will participate in the NCI SEER program (see individual project report NCI-4254). All field offices will be closed by August 31. Core staff are continuing to work on special projects such as the census tracting of all data collected.

During the coming year NCI staff will continue the analysis of the total data. A second monograph is planned to contain incidence data by socioeconomic class, cancer incidence among those of Spanish origin, geographic variation of cancer incidence and incidence of skin cancer in the United States. A third

monograph will contain information of treatment, length of hospital stay and costs. In addition a series of papers on individual sites of cancer is planned. Some of these may involve collaboration with principal investigators listed on the following page.

Date Contract Initiated: See table on following page.

Current Contract Level: See table on following page.

Contractor	Director	Initiated	Period	Level
University of Alabama	Dr. Harold Schnaper	10/ 1/68	9/ 1/73- 8/31/74	$ 13,010
Colorado Department of Public Health	Dr. Vernon Wohlauer	10/ 1/68	9/ 1/72- 6/30/74	-
Puerto Rico Department of Public Health	Dr. I. Martinez	12/ 9/68	12/ 9/72- 3/31/74	30,000
Medical Association of Georgia	Dr. Gordon Barrow	10/ 1/68	9/ 1/73- 6/30/74	3,709
University of Minnesota	Dr. Leonard Schuman	10/ 1/68	9/ 1/72- 8/31/74	-
University of Pittsburgh	Dr. Carol Redmond	10/ 9/68	9/ 1/73- 8/31/74	49,540
University of Texas	Dr. Eugene Frenkel	10/ 1/68	9/ 1/73- 8/31/74	6,030
Mayo Foundation	Dr. Leonard Kurland	12/22/69	12/22/72-12/31/73	

CONTRACT NARRATIVE
BIOMETRY BRANCH, DCCP
Fiscal Year 1974

END RESULTS SECTION

CALIFORNIA DEPARTMENT OF PUBLIC HEALTH (CP3-3353)
CHARITY HOSPITAL OF LOUISIANA (CP4-3259)
UNIVERSITY OF CHICAGO (CP4-3210)
CONNECTICUT STATE DEPARTMENT OF PUBLIC HEALTH (CP4-3203)
INDIANA UNIVERSITY HOSPITAL (CP4-3205)
UNIVERSITY OF IOWA (CP4-3204)
MASSACHUSETTS HEALTH RESEARCH INSTITUTE(CP2-2077)
UNIVERSITY OF MICHIGAN (CP4-3258)
UNIVERSITY OF VIRGINIA (CP4-3206)
NEW YORK MEDICAL COLLEGE (CP3-3321)
SASKATCHEWAN, CANADA (REGINA)(CP3-3350)

Title: End Results Evaluation

Project Officer (NCI): Dr. Sidney J. Cutler

Objectives: To obtain and analyze data on cancer incidence, survival and mortality in order to study:

1. variation in incidence and/or mortality with respect to geographic and demographic characteristics,
2. changes over time in the use of different methods of therapy and diagnostic criteria,
3. the relative effectiveness of various treatment modalities as judged by patient survival, and any changes over time in survival.

To organize special studies to investigate in more detail findings revealed by analysis of the routinely collected data.

Methods Employed: The National Cancer Institute is sponsoring a Collaborative Program for Cancer Surveillance, Epidemiology and End Results Reporting (SEER Program). Participants in this program include both population-based and medical center registries so that both incidence data and survival data can be based on as broad a cross-section of the U.S. population as possible. The Biometry Branch has professional and technical responsibility for supervising these contracts. Information on individual patients is submitted to the Biometry Branch, according to a prescribed schedule, and in accordance with a uniform code. This program makes available to the National Cancer Institute information on a very large series of patients in a form which permits maximum flexibility in handling and analyzing the data, and also provides an extensive resource for special studies on selected series of cases. Partial support for the population-based registries and for registries in medical center hospitals is provided through the contract mechanism to make it possible

for the participating registries to meet specified criteria regarding completeness, currency and accuracy of information as well as to promote epidemiological investigation.

Significance to NCI Program and Bio-Medical Research: Information on incidence, survival and mortality from a representative cross-section of the U.S. population is required on a continuing basis so that the nature and magnitude of the cancer problem including changes over time can be determined. Through close scrutiny of variation in cancer incidence and survival with respect to geographic and demographic characteristics of the population and differential changes over time, specific etiologic hypotheses will emerge which when tested via special study mechanism should then lead to the identification of controllable risk factors. Such research is an integral part of the ultimate goal of the National Program: the reduction in the occurrence of and mortality due to cancer.

Major Findings: An analysis of data on 18 forms of cancer for 219, 493 white and 21,088 black patients diagnosed during the 10-year time period 1955-64 indicated that the level of survival for black patients was lower than that for white patients and for both races women had a higher level of survival than men. In general, the largest difference in the level of survival between whites and blacks was observed for the forms of cancer with the better prognosis such as cancers of the bladder, larynx and uterine corpus. For the more lethal forms, such as stomach and lung cancer, there were small differences in survival. The 5-year relative survival rates for black and white cancer patients, for all stages and for localized disease, are shown in the following table.

	5-Year Relative Survival Rate			
	All Stages		Localized	
	White	Black	White	Black
Corpus	72	40	83	63
Female breast	62	47	84	77
Cervix (invasive)	60	47	79	78
Bladder	56	28	69	47
Larynx	55	45	73	59
Prostate	51	41	64	58
Colon	46	36	73	66
Rectum	40	30	64	60
Hodgkin's disease	39	26	-	-
Kidney	36	40	62	69
Ovary	32	28	72	74*
Brain and cranial meninges	28	28	30	30
Chronic leukemia	27	19	-	-
Stomach	12	10	39	42
Multiple myeloma	9	9	-	-
Lung and bronchus	8	6	30	23
Esophagus	3	2	11	6
Pancreas	2	2	7	5
Acute leukemia	1	2	-	-

* Rate has a standard error between 5 and 10 percent.

Proposed Course of Project: Continued attention will be given to reducing the time lag in reporting incidence data and in reporting survival data for disease entities for which therapeutic practice is changing rapidly, e.g., leukemia and Hodgkin's disease. Greater emphasis will be placed on developing additional population-based registries to provide a resource for a wide variety of epidemiologic and clincial studies.

	Date Initiated	FY-74 Negotiated
CALIFORNIA DEPARTMENT OF PUBLIC HEALTH (CP3-3353)	7-1-62	784,655
CHARITY HOSPITAL OF LOUISIANA (CP4-3259)	10-1-63	46,778
UNIVERSITY OF CHICAGO (CP4-3210)	7-1-72	46,340
CONNECTICUT STATE DEPT. OF PUBLIC HEALTH (CP4-3203)	6-1-63	276,041
INDIANA UNIVERSITY HOSPITAL (CP4-3205)	7-1-63	78,640
UNIVERSITY OF IOWA (CP4-3204)	7-1-63	37,175
MASSACHUSETTS HEALTH RESEARCH INSTITUTE (CP2-2077)	7-1-63	-
UNIVERSITY OF MICHIGAN (CP4-3258)	10-1-72	40,614
UNIVERSITY OF VIRGINIA (CP4-3204)	7-1-66	43,674
NEW YORK MEDICAL COLLEGE (CP3-3321)	6-24-71	46,537
SASKATCHEWAN, CANADA (REGINA) (CP3-3350)	6-21-72	14,270

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

End Results Section

KAISER FOUNDATION RESEARCH INSTITUTE (NO1 CP 43283)

Title: A Study of Time Trends in Maternal X-Ray Exposures

Contractor's Project Director: Dr. Gary Friedman

Project Officer (NCI): Ms. Ardyce Asire

Objectives: The objective of this study is to determine whether changes have occurred over time in the frequency of diagnostic X-ray exposures to pregnant women. The area of major interest is Alameda County, California, because of the availability of leukemia incidence data for the same area.

Methods Employed: Fifty mothers per year will be selected randomly from delivery room log books that have been kept in the Kaiser Foundation Hospital since 1947. The medical chart of each mother selected will be reviewed and abstracted. Data will be checked and edited and transferred to computer tape for analysis. Data analysis will involve simple counts and graphic displays of the number of X-rays received each year, plus more sophisticated analyses to detect time trends.

Major Findings: This is a new contract and no results are available yet.

Significance to NCI Program and Biomedical Research: This study will produce the first substantive data to indicate whether there has been a decline in the use of diagnostic radiography on pregnant women over the past decade or so, as has been assumed by many people. Such a change may be related to the reduced incidence of acute lymphocytic leukemia in children during the past decade.

Proposed Course of Project: This study is expected to be completed by the end of two years.

Date Contract Initiated: February 19, 1974

Current Annual Level: $38,260

SUMMARY REPORT
EPIDEMIOLOGY BRANCH
JULY 1, 1973 THROUGH JUNE 30, 1974

The objective of the Epidemiology Branch is to test ideas concerning the origins of cancer through epidemiologic studies based on medical knowledge and experience and on an awareness of resources best available at the national or international level. An idea is developed from existing knowledge, which may come from the laboratory, the clinic, or other epidemiologic studies. Emphasis is placed on epidemiologic research based on important new information from laboratory investigations at the National Cancer Institute and elsewhere.

Staff Changes: Drs. William A. Blattner, Thomas W. Pendergrass and Richard B. Everson replaced Drs. Gordon W. Grundy and Edward T. Creagan as Staff Associates.

Outside Training: Dr. John J. Mulvihill completed his pediatric residency at the Johns Hopkins Hospital.

Research Program:

International Activities:

During the year the Branch was especially active in conducting and planning international research. Emphasis was placed on initiating or perpetuating resources abroad that do not exist in the United States.

Atomic Bomb Casualty Commission (ABCC):

A contract, in its second year, with the National Academy of Sciences-National Research Council, supports and extends cancer research at the ABCC clinics in Hiroshima and Nagasaki. The contract provides for continuation of current studies to determine if cancer mortality rates will continue to increase as the cohort ages, especially among persons exposed before they were 10 years old (as early results suggest), and to initiate programs to increase appreciation in use of ABCC as a resource by university-based Japanese physicians and statisticians. The program provides for several Japanese faculty members to spend one year at ABCC conducting their own research under the guidance of the regular staff and the Epidemiology Branch, NCI. The first appointee has completed his year at ABCC and has assumed a position as Associate Professor of Preventive Medicine in the new medical school at Kawasaki University. He was favorably impressed by the program, so we were able to recruit four visiting scientists for the current year -- from Tokyo University, Kyushu University, Hiroshima University and the National Institute of Industrial Health. One of these four will continue for an additional year, and the other three will be replaced by visiting scientists from Tokyo University and Hiroshima University. Under the contract, a tumor-tissue registry has been established in Hiroshima and will begin in Nagasaki in September 1974, to improve cancer surveillance in both communities.

International Union Against Cancer (UICC):

Under the aegis of the UICC's Committee on Cancer in Children, the Epidemiology Branch of NCI is collecting by mail data from pediatric centers throughout the world for an international comparison of childhood cancer according to cell type. The purpose is to determine if the relative frequencies of certain childhood cancers are inordinately high or low in specific locales. The response has been most generous. More than 60 centers have contributed abstracts on series of patients, giving the diagnoses by cell type. More than 40,000 abstracts have been accessioned. Each contributor receives within a week a tabulation of his data according to a standard format, with comments as to how the relative frequencies of cancers that he has seen differ from the experience elsewhere. In this way he can identify areas of special research interest within his own locale and publish his findings as he sees fit. When unusual occurrences of tumors of specific types are noted in a particular center, histologic specimens are requested for review under an NCI contract with the Albany Medical College. A summary report of the findings from all centers will be presented to the UICC in 1974 (34).

In October 1973 a Study Group on Childhood Cancer was convened in Amsterdam to review progress to date and plans for the future (32). Of the 21 recommendations made by a similar group under the sponsorship of WHO in December 1972, 4 were thought to be immediately feasible either within nations or internationally through the International Society of Pediatric Oncology. These 4 recommendations concerned registries for children 1) with multiple tumors, 2) with adult-type neoplasms, 3) with sibs who have developed cancer, and 4) with long-term survival to determine complications of therapy or genetic transmissibility of the tumor or associated malformations to the progeny. A fifth recommendation has already been implemented: establishment of a monthly Newsletter to speed communication to pediatric oncologists concerning important observations on the origins of childhood cancer.

Other International Activities:

Members of the Branch reported on newer understanding of the causes of cancer through epidemiology at the Annual Meeting of the Japan Public Health Association (14) in Hiroshima, on the 21 recommendations of the WHO Expert Panel on Childhood Cancer at the Annual Meeting of the International Society for Pediatric Oncology in Amsterdam, on multiple primary neoplasms in relation to familial cancer (13) and on multiple primary malignancies in Connecticut over a 30-year period (55) -- both presentations made at the Fifth Perugia International Conference on Cancer, and on genetic determinants of cancer at an international symposium on epidemiology held in Yugoslavia (11). In addition, one staff member contributed the chapter on human observations in an IARC monograph on the evaluation of carcinogenic risk of some inorganic and organometalic compounds (5).

A member of the Branch also served on the Panel on Environmental Mutagenesis and Carcinogenesis (U.S.-Japan Cooperative Medical Scientific Program) and on a newly established U.S.-Japan Cooperative Cancer Research Program.

Genetic and Familial Studies:

The Branch has increasingly become known for its interest in genetic determinants of cancer. The subject is important because it can identify persons at high risk of cancer who can be screened more frequently than usual for early detection of neoplasia and can be advised to avoid even small exposures to environmental agents known to induce cancer in man. During the year two comprehensive clarifications of current knowledge were prepared (11, 12), and several oral presentations made at hospitals and at the Annual Meeting of the American Association of Pathologists and Bacteriologists.

The aggregation in families of tumors of the same cell type or of certain dissimilar cell types are often referred by hospitals and practitioners to the Branch for evaluation and special study when indicated. In these studies the family history of neoplasia is carefully obtained, documentation for the diagnoses are secured when possible, physical examinations are performed, and batteries of laboratory tests are made using the most recently developed procedures at NCI and elsewhere. If feasible, physicians in the Branch conduct their examinations and obtain specimens in the field (i.e., at the local hospital), or if the procedures involved are beyond the capacity of facilities there, the family members are brought to NIH for study by our staff. These efforts have led to the delineation of several new family cancer syndromes, of value not only in studying the genesis of the tumors, but also in identifying family members in the line of descent, who can be advised to have frequent examinations for the earliest possible detection of cancer, which may be life-saving. During the past year reports have been published on familial gastric cancer and immunologic abnormalities (3), familial arrhenoblastoma and thyroid cancer (24), familial medullary thyroid carcinoma and pheochromocytoma (28). Of the families currently under study four or five are likely to lead to published reports.

Childhood Cancer:

Since 1961, the Branch has paid particular attention to the etiology of childhood cancers as revealed by epidemiology -- a subject little studied at that time. During the past year continued use has been made of the Branch's national registry of death certificates for all children who died of cancer in the United States, 1960-1969. Information from these certificates has been coupled with multihospital surveys in which hospital charts for children with a specific neoplasm are abstracted to gain information that is not available through death certificates. Among the findings made or published during the year are marked variations in mortality from childhood cancers according to cell type, age, sex and ethnicity (submitted for publication); differences in mortality from rhabdomyosarcoma according to anatomic site (47); and comprehensive evaluations of the epidemiologic (etiologic) characteristics of teratomas in children (which were found to be associated with pelvic anomalies) (15), non-Hodgkin's lymphoma in children (18), and ovarian cancers in childhood (26). In addition, death-certificate registries are being used to identify children born in military hospitals, so their maternal obstetric records can be reviewed for diagnostic x-ray exposures during pregnancy, as a further test of the claim that low prenatal doses induce leukemia and other cancers before ten years of age.

The Epidemiology Branch is assisting the Biometry Branch in preparing a report on childhood cancer using data from the Third National Cancer Survey. This resource offers the first population-based estimates of U.S. childhood cancer incidence according to cell type. (There have been no childhood cancer registries in the United States suitable for this purpose.)

The National Wilms' Tumor Survey, performed under an NCI contract with the American Academy of Pediatrics, has generated some data of etiologic interest. The rate of congenital absence of the iris of the eye in children with Wilms' tumor was confirmed to be 1 in 75 as compared with one in about 75,000 in the general population. Congenital hemihypertrophy was found to be substantially more frequent than previously appreciated (about 1 in 40) -- due to increased awareness of its association with Wilms' tumor. The previously established elevated frequencies of genitourinary defects and familial Wilms' tumor were confirmed. The results indicate the desirability of collaborative studies for other childhood cancers with specially tailored check-lists for each type of neoplasm to note its associated anomalies and the occurrence of other tumors in the patient and his family. It is also desirable to determine the extent to which children with neoplasia are treated by expert teams of physicians. Plans are underway to make these determinations, probably through a contract with the American Academy of Pediatrics.

Other reports on childhood cancer issued during the year included a chapter in a book (38) and two reports based on data resources other than those described above (50, 56). Other papers have been prepared on a mortality-morbidity study of soft-tissue tumors other than rhabdomyosarcoma (an excess of certain cancers was found in first-degree relatives), and on the generally less favorable survival of Black children than white children with acute lymphotic leukemia.

The Proceedings of a Meeting on The Susceptibility of the Fetus and Child to Chemical Pollutants, edited by a member of the Branch, were published in full (45) and summarized in Science (46). Two members of our group had main papers in the conference (9, 40).

Adult Cancers:

About nine years ago the Branch acquired a copy of a computer tape containing information from the death certificates for all persons who died of cancer in the United States since 1950. This resource has markedly increased in value with the recent added capability of determining mortality according to county, whereas formerly it could be determined only by state. The tapes now contain death-certificate information for about 5 million people who died of cancer in the United States, 1950-1969. The tapes were used during the past year to determine 1) that (as yet) there has been no change in cancer mortality in Duluth, Minnesota, which can be related to reports of asbestos-like fibers in the water supply (31), 2) an excess mortality from stomach cancer in 23 coal-mining counties, 1950-69, appeared to be associated with socioeconomic class, rather than with occupation (4), and 3) an excess mortality from brain tumors in Eastern Kentucky reported by other investigators was confirmed in a 6-county area, for males over 44 years of age, 1965-69, suggesting an etiologic role of as yet unknown environmental agents (2). Other studies in progress

concern counties with high rates for cancers of the bladder, stomach and lung in relation to possible industrial exposures. The computer tapes have also been used to study the characteristics of cancer mortality among Orientals living in the United States. In a study of the Chinese, 1950-69, there were 5713 deaths from cancer, with a significantly higher mortality rate than among U.S. white males. The corresponding rate among Chinese females was significantly lower than that for white females. Nasopharyngeal cancer was elevated 26-fold in Chinese males and 22-fold in Chinese females. Several other neoplasms also showed significant excesses or deficiencies among the Chinese as compared with the rest of the U.S. population (16). A study of cancers among Japanese-Americans is in progress, and one report has been published on the higher rates for Hodgkin's disease among Japanese living in the U.S. as compared with those in Japan (29).

A major achievement of the year was publication of a 729-page compendium of tables on cancer mortality by county and anatomic site for the U.S., 1950-69 (30). The data can be used at the national, state and local level to determine if cancer mortality by county differs from normal, and to test hypotheses about possible occupational or other environmental causes.

Use of VA hospital data is currently being negotiated. Since 1958 about 300,000 veterans have been treated at VA hospitals for cancer. Even rare tumors are numerous enough in this series to permit study of the medical charts and for entries of possible etiologic significance. Also, patients with specific surgical or medical procedures or diagnoses in the past can be evaluated for subsequent unusual cancer mortality or morbidity (example: the relation of sprue to lymphoma).

The VA hospital records will complement studies already in progress under a contract between the Branch and the Follow-up Agency of the National Academy of Sciences. The Agency has access to diagnoses and certain other data during military service, which makes record-linkage possible to determine how events during military service affect causes of death. During the coming year studies of special interest include 1) causes of death among veterans whose spleens were removed because of trauma during military service in World War II, 2) the relation of cancer mortality to use during the Korean War of adenovirus vaccine contaminated with SV40 (an oncogenic virus in experimental animals), and 3) the relation of childhood cancer deaths under 10 years of age to maternal abdominal diagnostic x-ray exposure during pregnancy, as revealed by linking our childhood death-certificate data with obstetric records for the 3% of the children born in military hospitals.

Immunologic factors in cancer etiology can be studied not only through the data of the VA hospitals and the Follow-up Agency, mentioned above, but also through three other resources. First, the registry of renal-transplant receipients maintained by the American College of Surgeons continues to be studied by the Branch for additional details concerning factors related to the marked excess of reticulum cell sarcoma (350 times normal). Second, the Branch will assume responsibility for the small component of a Virus Cancer Program contract which supports the International Registry of Patients with Immunodeficiency Diseases and Cancer. With guidance from the Branch, the risk of malignancy can be better quantitated in the future than it has been to date. Third, a

retrospective cohort study has been initiated to determine the health status of civilian employees of Fort Detrick in relation to a past record of "hyperimmunization," to protect them from laboratory infections.

The Boston Field Station continues to pursue and stimulate studies of unusual observations of possible etiologic significance in patients with cancer at the Children's Cancer Research Foundation. A study has been initiated there of long-term survivors to assay the complications of therapy and the health of the progeny. A 20-fold increase in second primary cancers has been found, due largely to radiotherapy. Other studies at the Field Station involve extensive laboratory tests in families with unusual aggregates of cancer.

The Branch is planning and co-sponsoring with the NCI Cancer Control Program and the American Cancer Society a conference on Persons at High Risk of Cancer, to be held in December 1974. The purpose is to define large excesses in risk with regard to host, environmental or demographic factors. The results of the Conference should be useful both in the prevention and detection of cancer.

Veterinary Medical Data Program (VMDP):

In 1961 the Epizoology Section was initiated in the expectation that research into neoplasia among domestic animals would provide new insight into the origins of human cancer. At that time, there was an absence of systematically collected data for epidemiologic research. The Section developed a procedure for making standard observations on a mass scale to permit machine-processing and retrieval. Thirteen veterinary colleges contribute about 14,000 abstracts per month summarizing diagnostic, demographic and identifying information on each animal discharged. Each contributing institution receives a monthly listing of its data; NCI has the pooled data for its use. Since its beginning, the VMDP has accumulated more than 1 million abstracts, including 20,000 tumors reported during the period March 1964 through December 1972. The reports issued during the year described the possible relation of feline infectious anemia to subsequent leukemia (53), that certain breeds of dogs were at high risk of chemodectoma (21), and the epidemiologic characteristics of pancreatic carcinoma in animals (52). Studies of diseases other than cancer have shown that congenital umbilical and inguinal hernias are especially prevalent in certain species and breeds (20), that lead poisoning is more prevalent in cattle than other species (54), and that Miniature and Toy Poodles are especially prone to canine progressive retinal atrophy (51).

Epidemiology Branch

1. Barnes, B. A., Bergan, J. J., Braun, W. E., Fraumeni, J. F., Jr., et al: Eleventh report of the human renal transplant registry. JAMA 226: 1197-1204, 1973.

2. Creagan, E. T. and Fraumeni, J. F., Jr.: Deaths from brain tumors in Eastern Kentucky, 1950-69. J. Natl. Cancer Inst. 51: 1717-1718, 1973.

3. Creagan, E. T. and Fraumeni, J. F., Jr.: Familial gastric cancer and immunologic abnormalities. Cancer 32: 1325-1331, 1973.

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9. Fraumeni, J. F., Jr.: Chemicals in human teratogenesis and transplacental carcinogenesis. Pediatrics (Suppl.) In press.

10. Fraumeni, J. F., Jr.: Family studies in Hodgkin's disease. Cancer Res. In press.

11. Fraumeni, J. F., Jr.: Genetic determinants of cancer. In Doll, R. and Vodopija, I. (Eds.): IARC Monograph on Host-Environment Interactions in the Etiology of Cancer in Man. IARC Scientific Publ. No. 7, Lyon. In press.

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13. Fraumeni, J. F., Jr.: Multiple primary neoplasms: relationship to familial cancer. In Proc. Fifth Perugia Quadrennial International Conference on Cancer: Multiple Primary Malignant Tumors. In press.

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17. Fraumeni, J. F., Jr. and Mulvihill, J. J.: Persons at high risk of colorectal cancer. In Schottenfeld, D. (Ed.): Cancer Epidemiology and Prevention: Current Concepts. Springfield, Ill., Charles C. Thomas, In press.

18. Grundy, G. W., Creagan, E. T. and Fraumeni, J. F., Jr.: Non-Hodgkin's lymphoma in children: epidemiologic features. J. Natl. Cancer Inst. 51: 767-776, 1973.

19. Grundy, G. W. and Uzman, B. G.: Breast cancer associated with repeated fluoroscopy. J. Natl. Cancer Inst. 51: 1339-1340, 1973.

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43. Miller, R. W.: Radiation-induced cancer. In Schottenfeld, D. (Ed.): Cancer Epidemiology and Prevention: Current Concepts. Springfield, Ill., Charles C. Thomas. In press.

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45. Miller, R. W. (Ed.): The Susceptibility of the Fetus and Child to Chemical Pollutants. Pediatrics Supplement, May 1974. In press.

46. Miller, R. W.: The susceptibility of the fetus and child to chemical pollutants. Science. In press.

47. Miller, R. W. and Dalager, N. A.: Fatal rhabdomyosarcoma among children in the United States, 1960-69. Cancer. In press.

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55. Schoenberg, B. S., Fraumeni, J. F., Jr., Greenberg, R. A. and Christine, B.: Multiple primary malignancies in Connecticut, 1935-1964: clues to etiology. In Proc. Fifth Perugia Quadrennial International Conference on Cancer: Multiple Primary Malignant Tumors. In press.

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obert W. Miller, M.D.

Conferences

National Health Foundation, Conference on Cancer Epidemiology. New York, New York. August 16, 1973.
DBS Advisory Council, Boston Drug Surveillance Group's paper on maternal Salk vaccination during pregnancy as a cause of childhood cancer. August 31, 1973.
UICC Study Group on Childhood Cancer. Co-Chairman and rapporteur. Amsterdam, The Netherlands. October 3, 1973.
Advisory Committee on Epidemiology and Veteran's Studies, NAS-NRC Member. October 24, 1973.
Committee on Environmental Hazards, American Academy of Pediatrics, Chairman. Evanston Illinois, November 2-3, 1973; Bethesda, Maryland March 29-30, 1974.
U.S.-Japan Panel on Environmental Mutagenesis and Carcinogenesis. Charleston, South Carolina, November 18-21, 1973; Bethesda, Maryland, January 4, 1974; Bethesda, Maryland, April 2, 1974.
Ad hoc study section on sudden infant death syndrome. NIH-DRG. January 9, 1974.
Institut de la Vie. Workshop on teratology. Participant. Guadeloupe, FWI. January 24-29, 1974.
Symposium on Adverse Effects of Phototherapy, NICHD. Participant. Bethesda, Maryland. April 24-26, 1974.
Advisory Committee for Pediatric Oncology, Children's Hospital of Philadelphia. Member. Philadelphia, Pennsylvania. April 28-29, 1974.
U.S. Advisory Committee, U.S.-Japan Cooperative Cancer Research Program. Member. Honolulu, Hawaii. May 14-15, 1974.
WHO Expert Committee on Evaluation of and Guidelines for Programmes for the Early Detection of Cancer. Geneva, Switzerland. June 24-29, 1974.

Consultant

Children's Hospital of Philadelphia. Carcinogenesis Area contract on late effects of chemotherapy on long-term survivors of childhood cancer. Philadelphia, Pennsylvania, September 20, 1973.
Committee on Nutrition. American Academy of Pediatrics. Evanston, Illinois, November 1, 1973.
Committee on Neoplastic Diseases. American Academy of Pediatrics. Evanston, Illinois, November 9, 1973.
Review Committee to determine if CPSA ban on spray adhesives should be lifted. January 1974.

Lectures

"Late effects of radiation," American Bar Association, Committee on Radiation. Bethesda, Maryland, September 22, 1973.
"Epidemiology of leukemia," NCI Pediatric Rounds. Bethesda, Maryland, September 25, 1973.

"Etiology of childhood cancer," Manchester Paediatric Club. Manchester, England, October 1, 1973.
"The 21 recommendations of a WHO group consultation on research and care in childhood cancer," International Society for Pediatric Oncology. Amsterdam, The Netherlands, October 6, 1973.
"Cancer and congenital malformations," Pediatric Ground Rounds. Johns Hopkins Hospital, Baltimore, Maryland, November 10, 1973.
"Genetics of cancer," Symposium at Annual Meeting of American Association of Pathologists and Bacteriologists. San Francisco, California, March 11, 1974.
"Environmental causes of childhood cancer," ACS Conference on Childhood Cancer. Dallas, Texas, May 16, 1974.

Editorial Committee

Fogarty Center Preventive Medicine Program: Book on Prevention of Perinatal Diseases. November 13, 1973.

Associate Editor

Teratology Journal
Journal of the National Cancer Institute

Joseph F. Fraumeni, Jr., M.D.

Committee Membership

Chairman, Panel on Chronic Disease Epidemiology, United States-United Kingdom Cooperative Program in Envrionmental Health Sciences.
Chairman, NIH Committee on Transplantation Research.
Cause and Prevention Executive Staff, National Cancer Institute.
Field Studies and Statistics Program Review Group, National Cancer Institute.
Scientific Advisory Committee, Cancer Control Bureau, New York State Department of Health.
Cancer Research Training Committee, National Cancer Institute.
Epidemiology Committee, Breast Cancer Task Force, National Cancer Institute.
Advisory Committee, American College of Surgeons Renal Transplant Registry.
Program Committee, American Association for Cancer Research.
Editorial Advisory Board, Cancer Research.
Editor, Medical and Pediatric Oncology.
Committee on Cancer Prevention and Detection, American Society of Clinical Oncology.
Smoking and Health Review Board, National Cancer Institute.

Consultant

Atomic Bomb Casualty Commission, Hiroshima, Japan, October, 1973.

Lectures

"Epidemiologic Studies of Immunosuppression and Cancer," National Cancer Advisory Board Meeting. Bethesda, Maryland, October 3, 1973.
"People with Special Susceptibility to Cancer: Etiologic Clues," Cancer Epidemiology Course, School of Public Health, Hawaii University, and Staff Conference, St. Francis Hospital, Honolulu, October 5, 1973.
"New Understanding of the Causes of Cancer by Epidemiology," Japan Public Health Society, Annual Meeting. Hiroshima, Japan, October 12, 1973.
"Pancreatic Cancer: Epidemiologic Considerations," National Cancer Advisory Board Meeting. Bethesda, Maryland, November 26, 1973.
"Genetic and Immunological Determinants of Cancer: Epidemiologic Evidence," University of Miami Cancer Center. Miami, Florida, December 18, 1973.
"Host Susceptibility to Cancer," Children's Hospital of Philadelphia. Philadelphia, Pennsylvania, January 23, 1974.
"Cancer Mortality among Chinese Americans," American Association for Cancer Research, 65th Annual Meeting. Houston, Texas, March 29, 1974.
"Persons at High Risk of Leukemia and Lymphoma," National Conference on Acute Leukemia. Montreal, Canada, June 6, 1974.

illiam A. Priester, DVM

Conferences

Carcinogenesis Program, NCI, Information and Resources Segment. Conference on Data Dissemination for Carcinogenesis. Williamsburg, Virginia, January 30, 31, and February 1, 1974.

Lectures

"The Veterinary Medical Data Program--Current Status Report," Annual Meeting, American Veterinary Medical Association. Philadelphia, Pennsylvania, July 16, 1973.
"Quality Control in Veterinary Records," Annual Meeting, American Association of Veterinary Medical Records Administrators. Philadelphia, Pennsylvania. July 18, 1973.

ohn J. Mulvihill, M.D.

Committee Membership

National Academy of Sciences-National Research Council
Committee on the Biological Effects of Ionizing Radiation
Subcommittee on Growth and Development
American Academy of Pediatrics
Committee on Environmental Hazards

oward M. Hayes, DVM

Lectures

"The Use of the Veterinary Medical Data Program in Cancer Epidemiology,"

Armed Forces Institute of Pathology, Washington, D. C., November 5, 1973.

Frederick P. Li, M.D.

Committee Memberships

Board of Trustees, Boston Tuberculosis and Respiratory Disease Association.
Biometry and Epidemiology Contract Review Committee, National Cancer Institute.
Committee on Professional Education for Cancer Control, National Cancer Institute.

Robert N. Hoover, M.D.

Committee Memberships

The ad hoc committee for review of unsolicited proposals to the cancer control program.

Consultant

Children's Hospital of Philadelphia. Carcinogenesis Area contract on late effects of chemotherapy on long-term survivors of childhood cancer. Philadelphia, Pennsylvania, September 20, 1973.

Conferences

American Public Health Association. Annual Meeting. San Francisco, California, November 4-8, 1973.
National Academy of Sciences, "Statistics in the Environment." Washington, D.C., March 6-7, 1974.

Lectures

"Industrial Bladder Cancer," Japanese National Health Systems Study Team. NIH, Bethesda, Maryland, June 11, 1973.
"Epidemiology of Bladder Cancer," Symposium on Chemical Carcinogenesis, Industrial Health Foundation at the Mellon Institute. Pittsburgh, Pennsylvania, June 28, 1973.
"Risk of Cancer in Renal Transplant Recipients," Medical College of Wisconsin. August 1973.
"Non-familial Epidemiologic Considerations in Breast Cancer," Workshop on Genetics and Human Breast Cancer. Breast Cancer Task Force, NIH, Bethesda, Maryland, September 10, 1973.
"Risk of Cancer in Renal Transplant Recipients," Seminar at UCLA. California, February 27, 1974.

Serial No. NCI-4325
1. Epidemiology Branch, OADFS&S, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Planning and Development in Cancer Epidemiology

Previous Serial Number: Same

Principal Investigator: Robert W. Miller, M.D.

Other Investigators: J. F. Fraumeni, Jr., M.D., W. A. Priester, DVM, and other professional staff of the Epidemiology Branch

Cooperating Units: None

Man Years:

Total	:	2.0
Professional	:	2.0
Other	:	0.0

Project Description

Objectives:

1. To originate new epidemiologic approaches to the study of cancer causation in man and animals.

2. To develop sources of data related to specific epidemiologic problems.

3. To stimulate epidemiologic research in other health agencies.

4. To develop on-the-job training for medical and statistical officers.

Methods Employed:

The Program is based on:

1. Leads from animal experimentation, laboratory research and clinical observations.

2. Prospective studies (in retrospect) which relate cancer occurrence to events recorded prior to the onset of cancer in medical examinations obtained in a standard fashion from large numbers of persons (examples -- clinical health-surveys, and military medical examinations).

3. Retrospective studies based on questionnaires obtained by personal interview or by mail, for a comparison of persons (examples -- clinical health-surveys, and military medical examinations).

4. "Laterospective" studies which concern the detection from clinic records of the excessive concurrence of cancer with pre-existent disease, such as congenital defects or autoimmune disorders.

As specific epidemiologic questions arise from laboratory or clinical observations, sources of field data are developed to answer these. Conversely, the Branch seeks by its surveys to raise questions which can be answered by laboratory or clinical studies. Parallel with this, to fill the tremendous deficiency in qualified personnel, the selection and training of young physicians, veterinarians, and statisticians must take place. The principles they learn from the guided planning, execution, and analysis of a program in cancer epidemiology are applicable to studies of the genesis of congenital malformations, heart disease, arthritis, mental aberrations, or the effects of ionizing radiation.

Significance to Bio-medical Research and the Program of the Institute:

If the example set by the Branch is a good one, interest will be stimulated among medical scientists in the use of epidemiologic methods for their research. In consequence, there may develop a further recognition of the usefulness of office and hospital records for survey studies. With this would go an appreciation for the need to adapt the standard medical records for epidemiologic research. These developments, in turn, could promote an interest in looking beyond the walls of hospitals and medical centers for opportunities in medical research.

Proposed Course of Project:

To develop opportunities in cancer epidemiology as they arise. During the past year an "Alert Practitioner Program" was initiated at the Boston Field Station in conjunction with similar NIEHS-sponsored feasibility studies in the Pediatrics Departments at Montefiore Hospital (New York City), Duke University and Los Angeles County-USC Medical Center. Under faculty supervision, medical students obtain special etiological histories and pedigrees from parents whose children have cancer or other diseases of unknown etiology. The purpose is to increase attention to etiology in medical schools, to seek new clues to causes of disease and to identify students with an aptitude to think epidemiologically, who can then be advised as to further training in this regard.

Serial No. NCI-4371
1. Epidemiology Branch, OADFS&S, DCCP
2. Epizoology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: National Cancer Institute Veterinary Medical Data Program

Previous Serial Number: NCI-4371

Principal Investigator: W. A. Priester, DVM

Other Investigators: H. M. Hayes, DVM

Cooperating Units: School and Colleges of Veterinary Medicine: Michigan State University, University of Missouri, University of Minnesota, Iowa State University, Ontario Veterinary College, Purdue University, University of Georgia, Kansas State University, University of Illinois, University of California, Ohio State University, University of Saskatchewan, Colorado State University

Also, see Project Report NCI-4380.

Man Years:

Total	:	2.40
Professional	:	.40
Other	:	2.00

Project Description

Objectives:

To provide animal disease data as a research resource. To coordinate the program with related programs primarily in area of disease nomenclature, terminology and code. To develop ways and means of data utilization applicable to comparative medical studies.

Methods Employed:

Standard data processing methods. Details are described in related Project Reports and Contract Narratives of Epizoology Section.

Major Findings:

Not applicable as this is primarily a service activity. The National Cancer Institute Veterinary Medical Data Program is currently processing data from 11 veterinary colleges in this country and 2 in Canada. The program has been endorsed and implemented in Europe under the auspices of WHO. For the first time, veterinary medicine and the medical sciences have benefit of a national and international animal disease data system. A number of cooperative studies were begun during the year with faculty members at the various participating colleges of veterinary medicine.

Significance to Bio-medical Research and the Program of the Institute:

The comparative (veterinary) medical data retrieval system instituted at the College of Veterinary Medicine, Michigan State University on a pilot basis (PH 43-64-85) was undertaken to design a system for collection, storage and retrieval of veterinary medical clinical data. The program is now fully operative and has been extended to 12 additional veterinary colleges. Establishment and maintenance of reliable sources of veterinary medical data provide a research resource, hitherto unavailable, that is applicable not only to cancer research but to research areas such as infectious diseases, congenital defects, and gerontological diseases. Continued NCI Epidemiology Branch animal studies research function depends upon continued availability of valid domestic animal disease data, particularly as regards neoplastic disease. The data bank, which included information on more than 1,000,000 patients through 1973, is often used as a reference source by scientists at NCI and elsewhere.

Proposed Course of Project:

Additional veterinary schools will be added to the program as interest and capability of the individual applicants are established. It is anticipated that this project will continue for a minimum of two years.

Date initiated: October 22, 1965

Current annual level: $34,000

Publications

Hayes, H. M. and Priester, W. A.: Feline infectious anemia. Risk by age, sex, and breed; prior disease; seasonal occurrence; and mortality. J. Small Anim. Pract. In press.

Priester, W. A. and Hayes, H. M.: Lead poisoning in cattle, horses, cats and dogs, as reported by 11 colleges of veterinary medicine in the United States and Canada from July 1968 through 1972. Am. J. Vet. Res. In press.

Priester, W. A.: Canine progressive retinal atrophy: occurrence by age, breed, and sex. Am.J. Vet. Res. In press.

Serial No. NCI-4377

1. Epidemiology Branch, OADFS&S, DCCP
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Familial Aggregation of Malignancies

Previous Serial Number: Same

Principal Investigators: Joseph F. Fraumeni, Jr., M.D. and Robert W. Miller, M.D.

Other Investigators: John J. Mulvihill, M.D., Frederick P. Li, M.D., William A. Blattner, M.D., Thomas W. Pendergrass, M.D., and Richard B. Everson, M.D.

Cooperating Units: Various NCI clinical and experimental groups

Man Years:

Total	1.50
Professional	1.25
Other	.25

Project Description

Objectives:

To document the occurrence of patterns of family aggregation of neoplasms. To study such families by genetic and laboratory investigations, in an effort to elucidate the degree to which heredity and/or the common familial environment contribute to the etiology of neoplasms. To distribute tissue and blood specimens from such families to interested laboratory investigators, for etiologic study by cytogenetic, immunological, viral, and tissue culture methods. To identify genetic disorders predisposing to cancer.

Methods Employed:

Interview of cancer patients with respect to family occurrence of cancer and other disorders, as well as information on prior medical and environmental history; documentation of history by obtaining appropriate vital records and hospital charts; collection and distribution of biological specimens from such families; review of hospital records of series of patients with selected genetic diseases or neoplasms; survey of childhood cancer death certificates in the U.S. since 1960 to identify sib aggregation.

Major Findings:

A number of families have been studied with sites-specific cancers, such as familial lymphoma, breast cancer, ovarian cancer, chronic lymphocytic leukemia, chronic myelogenous leukemia, acute leukemia, cancer of the colon, stomach cancer, and osteogenic sarcoma. Other familial occurrences have represented multiple cancer syndromes, including familial adenocarcinomas of multiple sites, and the familial syndrome of sarcoma, breast cancer and other neoplasms. Many of the tumors which occur together in familial syndromes also coexist excessively in studies of multiple primary cancer, suggesting that a familial cancer syndrome may represent a "scattering" over the family tree of tumors which share etiologic influences. The results of laboratory studies of specimens obtained from members of various cancer families are being assembled.

Reports were published on a variety of familial occurrences: a) familial stomach cancer with laboratory evidence of autoimmunity (parietal cell antibodies) and cell-mediated immune deficiency (impaired lymphocyte transformation in vitro); b) a new familial syndrome linking ovarian arrhenoblastoma and thyroid adenoma; c) in a series of children with non-Hodgkin's lymphoma, an excess in siblings of lymphoma, leukemia and brain tumor; and d) a report of laboratory studies in a family with the medullary thyroid carcinoma -- pheochromocytoma syndrome. Also published was a review of evidence for genetic factors in the origin of human cancer.

Significance to Bio-medical Research and the Program of the Institute:

Epidemiologic surveys and detailed studies of families at high risk of cancer may help to detect environmental and genetic influences in carcinogenesis. In addition, identification of these families has therapeutic implications, enabling surveillance and early diagnosis of neoplasms.

Proposed Course of Project:

The same approach will be continued. New laboratory methods and epidemiologic clues from other sources will be incorporated into the project protocol as necessary.

Publications

Fraumeni, J. F., Jr.: Genetic factors. In Holland, J.F. and Frei, E., III (eds.): Cancer Medicine, Philadelphia, Lea and Febiger, 1973, pp. 7-15.

Creagan, E. T. and Fraumeni, J. F., Jr.: Familial gastric cancer and immunological abnormalities. Cancer 32: 1325-1331, 1973.

Jensen, R. D., Norris, H. J. and Fraumeni, J. F., Jr.: Familial arrhenoblastoma and thyroid adenoma. Cancer 33: 218-223, 1974.

Li, F. P., Melvin, K. E. W., Tashjian, A. H., Levine, P. H. and Fraumeni, J. F., Jr.: Familial medullary thyroid carcinoma and pheochromocytoma. J. Natl. Cancer Inst. 52: 285-287, 1974.

Serial No. NCI-4378
1. Epidemiology Branch, OADFS&S, DCCP
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: U. S. Cancer Mortality Survey

Previous Serial Number: NCI-4378

Principal Investigator: Thomas J. Mason, Ph.D.

Other Inverstigators: Joseph F. Fraumeni, Jr., M.D., Frank W. McKay, Jr., and Robert N. Hoover, M.D.

Cooperating Units: National Center for Health Statistics

Man Years:

Total	:	3.25
Professional	:	2.75
Other	:	0.50

Project Description

Objectives:

To examine the cancer mortality experience in the United States, 1950-69 relative to cancer etiology for specific races, White, Black, American Indian, Chinese, Japanese, other, and also for individual counties within states.

Methods Employed:

Computer analysis of over 6 million death certificates by site, sex, race, state, and age. The investigation is ongoing, updated year by year, and expanding. Base populations for Whites, Blacks, and all nonwhites are maintained for the entire time period. The system for the total U.S. provides race, sex, ICD, and age specific analyses of cancer mortality consisting of numbers dead, populations at risk, age-specific and age-adjusted rates, a measure of the variability in the age-adjusted rate, and a linear regression of the age-adjusted rates as a function of time. This system is fully operational.

A system for the analysis of cancer mortality for individual counties with the flexibilities of the national system is currently operational.

Major Findings

The publication of cancer mortality rates by site, race, and sex for individual counties in the contiguous United States. The mortality data in this report may be used for various epidemiologic purposes. It should be possible to generate hypotheses to the origin of certain cancers by identifying counties and groups of counties with high rates, and determining if these areas share certain demographic or environmental variables. In approaching from the opposite direction, it should be possible to evaluate ideas or hypotheses by identifying counties with unusual environmental or demographic characteristics, and determining whether these areas have peculiarities in the frequency of particular cancers.

The completion of a random access demographic file with information on individual counties to facilitate etiologic investigations. Some of the variables included are: percent urban, percent rural, median school years completed age 25 or greater and percent nonwhite.

An investigation of cancer mortality in Duluth, Minnesota, where asbestos-like fibers in industrial waste first entered the water supply in 1955, found no apparent carcinogenic effect. Cancer mortality among persons of all ages and among children was considera

sexes, but significant only in males. On the other hand significantly low mortality was reported among Chinese males for prostate and bladder cancers, and among Chinese females for breast and cervical cancers. Mortality from cancers of the large intestine and rectum in Chinese was excessive in males but deficient in females.

Hodgkin's disease among Japanese Americans was investigated. The age-specific mortality in U.S. Japanese was somewhat higher than in Japan up to age 50, and substantially higher after age 65 to exceed the rates in the U.S. White population. Age-adjusted mortality rates were compared for the age groups 15-34, 35-49, and 50+ between the native and U.S. Japanese. The excess mortality in U.S. Japanese was statistically significant for age groups 15-34 and 50+. The shifts in mortality among U.S. Japanese toward the rates prevailing in the White population are consistent with an environmental influence at all ages, and do not support the notion that the young disease has distinctive characteristics of an infectious process.

To evaluate a previous report of excess brain tumor frequency in Eastern Kentucky, 1963-69, cancer mortality from these neoplasms were analyzed in a 6-county area for the time period 1950-69. Based on State rates, excess risk was observed only for males over age 44 during 1965-69. Although a chance occurrence cannot be discounted, the observations suggest the selective

exposure of males to environmental agents capable of inducing intracranial neoplams.

Significance to Bio-medical Research and the Program of the Institute:

This survey provides a continually expanding data set against which specific etiological hypotheses about cancer can be tested. The capability of sub-division of the data set into specific racial and geographical subsets (specifically county level analysis) provides an opportunity to more critically test hypotheses regarding the etiology of cancer than was possible at an earlier point in time.

Proposed Course of Project:

The completion of software to facilitate computer generated maps of the distribution of cancer mortality by site, race, and sex for individual counties. This software will be utilized in the preparation of an atlas on cancer mortality with emphasis on etiology.

Epidemiologic investigations of primary hepatoma and gallbladder cancer by county as well as cancer mortality among Japanese Americans are ongoing.

Studies of the geographic distribution of cancer mortality controlling for such factors as percent urban and median school years are proposed. An analysis of the underlying relationship between cancer mortality by site has been completed. This manuscript is in preparation.

Publications

Mason, T. J., McKay, F. W.: U.S. Cancer Mortality by County: 1950-1969, DHEW Publication No. (NIH) 74-615, 1973.

Mason, T. J., McKay, F. W., Miller, R. W.: Cancer mortality, in relation to asbestos in the water supply of Duluth, Minnesota. JAMA. In press.

Fraumeni, J. F., Jr., Mason, T. J.: Cancer mortality among Chinese Americans, 1950-69. J. Natl. Cancer Inst. In press.

Mason, T. J., Fraumeni, J. F., Jr.: Hodgkin's disease among Japanese Americans. Lancet 1: 215, 1974.

Creagan, E. T., Fraumeni, J. F., Jr.: Death from brain tumors in Eastern Kentucky, 1950-69. J. Natl. Cancer Inst. 51: 1717-1718, 1973.

Serial No. NCI-4379

1. Epidemiology Branch, OADFS&S, DCCP
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of Congenital Defects in Domestic Animals

Previous Serial Number: NCI-4379

Principal Investigator: Howard M. Hayes, D.V.M.

Other Investigators: William A. Priester, D.V.M. and John J. Mulvihill, M.D.

Cooperating Units: See Project Report NCI-4371

Man Years:

Total	:	.40
Professional	:	.20
Other	:	.20

Project Description

Objectives:

To study the characteristics of patients with congenital defects for clues as to possible relationships to cancer in the same clinic-hospital population. To relate these findings to human beings.

Methods Employed:

From case summaries submitted to the Veterinary Medical Data Program (NCI-4371), series of animals with congenital defects are systematically analyzed in relation to the total number of animals seen in the same time period. A general survey of the findings has already been published for cases seen from March 1964 through January 1969, as was a study about congenital ocular defects and reports about dogs with cardiovascular defects, patellar dislocation and hip dysplasia. During this year a survey has appeared on congenital umbilical and inguinal hernias. Currently in progress are studies of canine ectopic ureter.

Major Findings:

Females were at high risk for congenital umbilical hernia in cattle, horses and dogs. In horses and pigs, males were at high risk for congenital inguinal hernia. In dogs, the Pekingese was at high risk for both defects singularly and concurrently, the Airedale Terrier had high risk for congenital umbilical

hernia, the West Highland White Terrier had high risk and mix breed dogs had low risk for congenital inguinal hernias.

Significance to Bio-medical Research and the Program of the Institute:

Experiments on animals are essential to progress in medical research. Traditionally, a small number of species is used for laboratory experimentation. Currently popular laboratory animals are generally poor models for genetic disease and congenital malformations in man, and the rarity of these individual conditions in the human population limits clinical research. Our project attempts to detect conditions spontaneously occurring in domestic animals which are more nearly homologous to human afflictions. Taking heart malformations in dogs as an example, we have defined high frequencies of 1) patent ductus arteriosus in Miniature/Toy Poodles, and 2) persistence of the right aortic arch in German Shepherds. Since certain congenital malformations in man are associated with childhood malignancies, we hope to identify domestic animals which may serve as research models to explore the borderland between teratology and oncology.

Proposed Course of Project:

Data will continue to come from the Veterinary Medical Data Program and will be analyzed for breed-specific lesions.

Publications

Hayes, H. M.: Congenital umbilical and inguinal hernia in cattle, horses, pigs, dogs, and cats: risk by breed and sex among hospital patients. Am. J. Vet. Res. In press.

Serial No. NCI-4380
1. Epidemiology Branch, OADFS&S, DCCP
2. Epizoology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Study of the zoographic characteristics of domestic animals with tumors.

Previous Serial Number: NCI-4380

Principal Investigator: W. A. Priester, DVM

Other Investigators: Howard M. Hayes, DVM

Man Years:

Total	1.40
Professional	.40
Other	1.00

Project Description

Objectives:

To provide a reference of the distribution of spontaneous tumors in domestic animals. To search for unusual distribution of tumors, within a large, animal hospital population, which might suggest possible clues regarding the etiology of cancer.

Methods Employed:

Using patients with tumors reported to the Veterinary Medical Data Program as case series, and all patients seen during like periods as population references, the following will be studied:

Characteristics of patients: age, breed/species, sex
Characteristics of tumors: degree of malignancy, site, and histogenic type.

In addition, other factors may be studied as the analyses progress.

Major Findings:

20,000 tumors were reported during the period March 1964 through December 1972 among 400,000 animal-years-at-risk. The proportion of patients with tumors was highest among dogs and lowest among horses, when the four major species

categories, bovine, equine, feline, and canine, were considered. In almost all of the studies of specific tumors conducted so far age has been generally associated with an increasing risk for tumors but not to the same degree for each specific tumor type. Invariably, one or more breeds of dogs, can be identified as being at excess risk for the specific tumor type under study. While no striking associations between specific tumor type and other primary tumors in the same patient have been noted, several associations were identified that seemed similar to conditions described for man.

Significance to Bio-medical Research and the Program of the Institute:

Animal studies done by the Branch have demonstrated several relationships of interest in cancer research -- bone sarcoma and size, canine lymphoma and certain breeds, canine mastocytoma and breed ancestry. This study is designed to detect high risk groups in a systematic fashion. The results may well define animal subgroups suitable for studies of cancer etiology that would be impractical in a human population.

Proposed Course of Study:

Data will be added to the tumor file and reference population base at the end of each calendar year. While some high-frequency tumors will be studied, the main effort will be directed towards study of those spontaneous tumors in domestic animals that have been so infrequently reported in the literature that characterization of patients with these tumors has never been done.

Date initiated: May 1970

Current annual level: $19,000

Publications

Priester, W. A. and Hayes, H. M.: Feline leukemia after feline infectious anemia. J. Natl. Cancer Inst. 51: 289-291, 1973.

Priester, W. A.: Pancreatic carcinoma in domestic animals. Data from 11 U.S. and Canadian colleges of veterinary medicine. Cancer Res. In press.

Hayes, H. M. and Fraumeni, J. F., Jr.: Chemodectomas in dogs: epidemiologic comparisons with man. J. Natl. Cancer Inst. In press.

Serial Number NCI-4400
1. Epidemiology Branch, OADFS&S, DCCP
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
June 1, 1973 through June 30, 1974

Project Title: Epidemiology Branch Field Station, Boston, Massachusetts.

Previous Serial Number: NCI-4400

Principal Investigator: Frederick P. Li, M.D.

Other Investigators: None

Man Years:

Total	:	1.8
Professional	:	1.0
Other	:	0.8

Project Description

Objectives:

1) To study the epidemiology of human neoplasms and to develop etiologic hypotheses through close observation of individual patients at the bedside.

2) To provide consultation in cancer epidemiology to staff physicians and trainees in the Boston area, and to stimulate therapists to observe and report clinical findings of etiologic significance.

Methods Employed:

Over 150 questionnaire interviews of childhood cancer patients have been completed, with the finding of several families with strong histories of cancer. Laboratory studies have been carried out in some families to identify possible inherited defects or environmental factors which predispose to cancer. In addition, a registry has been started of long-term survivors of childhood cancer. These patients and their progeny are being followed for development of new tumors and for evidence of late complications of cancer therapy. In the hematologic malignancies, 3 epidemiologic studies of Hodgkin's disease are in progress: association with dilantin ingestion, or with prior tonsillectomy, and effect of age on clinical features of the tumor.

Major Findings:

1) Twenty-five sibships with childhood cancer have been collected, revealing a high frequency of cancer at a relatively early age in parents and other cl relatives.

2) A 20-fold higher risk of a new cancer was found in childhood cancer survivors as compared with persons in the general population.

3) Nearly 100 offsprings of childhood cancer survivors show no evidence of genetically transmitted damage from cancer therapy employing potential mutagens.

4) A causal association between tonsillectomy and Hodgkin's disease could not be shown in a study of 150 cases and appropriate controls. In addition, a history of dilantin ingestion is uncommon in Hodgkin's disease patients, indicating that very few cases of the tumor can be attributed to this drug.

Significance to Bio-medical Research and the Programs at the Institute:

Studies at the Field Station generate etiologic hypotheses for clinical and laboratory testing. The studies also help evaluate the relevance for human populations of etiologic data provided by other investigators at the Institute. The teaching of cancer epidemiology provided to medical students and junior house staff may help to stimulate interest in cancer epidemiology and to influence the selection of a career in this field.

Proposed Course of Project:

The Field Station will continue to focus on studies of the epidemiology of childhood cancers. The emphasis will be on detection of clues to etiology through careful clinical study of the natural history of cancer in individual patients. The new questionnaire studies will hopefully provide clues to cancer etiology which were not evident from retrospective reviews of prior data sources. The registry of long-term survivors is unique and will provide data on the inheritance of childhood cancers, and on oncogenesis associated with prior cancer therapy.

Serial No. NCI-4401

1. Epidemiology Branch, OADFS&S, DCCP
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Immunologic Factors in Cancer Etiology

Previous Serial Number: None

Principal Investigator: Robert N. Hoover, M.D.

Other Investigators: Joseph F. Fraumeni, Jr., M.D.

Man Years:

Total	:	1.5
Professional	:	0.5
Other	:	1.0

Project Description

Objectives:

1) To identify populations with altered immunologic status who experience excessive rates of malignancy.

2) To study these populations in order to identify the characteristics and possible determinants of any excess risk.

Methods Employed:

In the past year we conducted a cohort study of 6297 renal transplant recipients who were reported to a renal transplant registry over the past 20 years. Age, sex, and time-specific numbers of person-years of post-transplant experience were calculated for this population. Cancer incidence rates from the general population were applied to these tabulations in order to develop expected numbers of cancers with which to compare the numbers actually observed.

A similar study was done of 500 transplant recipients whose graft failed and, therefore, were maintained on a renal dialysis program.

Major Findings:

1) The risk of lymphoma among renal transplant recipients is about 35 times greater than normal and derived almost entirely from a risk of reticulum cell sarcoma that was 350 times greater than expected.

2) The excess lymphoma risk appeared within a year of transplant and remains at the same high level for the 5 or more years of follow-up.

3) Skin and lip cancers occurred up to 4 times more often than expected.

4) Other cancers were 2.5 times more common, in men only, due largely to soft-tissue sarcoma and hepatobilliary carcinoma.

5) There was a suggestion that the risk of lymphoma was greater for those recipients whose age was widely different from their donor's and less for recipients with blood group O.

6) The excess lymphoma risk does not appear to extend to transplant recipients whose graft has failed and who have returned to dialysis.

Proposed Course of Project:

1) The same population of transplant recipients is being followed and will be re-evaluated yearly.

2) Several more detailed studies are currently underway utilizing the patients reported to this registry. Data is currently being collected for a case-control study of transplantation antigens and the characteristics of the "match" for patients who subsequently developed a malignancy. A study of the reproductive experiences of the transplant population is currently being planned among several of the larger transplant centers and the Branch.

3) A study of the occurrence of a virus induced neoplasm -- the common wart -- among transplant recipients and dialysis patients is currently being analyzed.

4) The Branch has entered into collaboration with a registry of cancer in children with immunodeficiency syndromes. Two goals will be pursued using this resource over the next year. An attempt will be made to quantify the risk of malignancy in this population. An attempt will also be made to identify the characteristics of those immunodeficient children who developed cancer vs. those who did not.

5) A number of studies relevant to this topic are currently being designed in collaboration with the central office of the Veteran's Administration. The initial studies will be retrospective cohort studies of patients with various immune-deficiency syndromes or autoimmune diseases and others undergoing procedures or drug therapies involving immunosuppression. A study with the VA of the frequency of malignancy in dialysis patients is also being planned.

6) Data is currently being collected for a retrospective cohort study of the former civilian employees of Fort Detrick (a biologic warfare research center). A large proportion of these workers fall into a group which would be considered "hyperimmunized."

Publications

Hoover, R. N. and Fraumeni, J. F., Jr.: Risk of cancer in renal-transplant recipients. Lancet 2: 55-57, 1973.

CONTRACT NARRATIVE
(Summary of Personal Service Contracts)
Fiscal Year 1974
Animal Studies

VETERINARY MEDICAL DATA PROGRAM

Personal Service Contracts between the Epizootiology Section and Colleges of Veterinary Medicine at the University of Missouri, Michigan State University, Iowa State University, Ontario Veterinary College, and the University of Georgia, University of Illinois, Kansas State University, Ohio State University, University of Saskatchewan, Purdue University, University of Minnesota, University of California, and Colorado State University.

Title: Purchase Veterinary Case Data.

Objectives: To add to volume of stored data, available to Epizootiology Section, in the Veterinary Medical Data Program. To further encourage adoption of standard nomenclature and coding of animal disease data. To make available to veterinary medicine, comparative medical research and the scientific community the developments of the Veterinary Medical Data Program.

Methods Employed: Purchase of completed veterinary medical case abstracts in terms of National Cancer Institute Veterinary Medical Data Program (as detailed in related Contract Narratives).

Major Findings: None, as this is primarily a service activity.

Significance to NCI Program and Bio-medical Research: Increase store of data in the Veterinary Medical Data Program. Of particular importance in terms of study of low prevalence diseases (cancer, etc.), geographical distribution and absolute numbers.

Proposed Course of Project: Minimum of two years. On the basis of a long-standing agreement, it is likely that the College of Veterinary Medicine, Auburn University, will be added to the program sometime during the coming fiscal year.

Dates Initiated:

Michigan State University	-	November 1965
Iowa State University	-	October 1965
Ontario Veterinary College	-	July 1966
University of Georgia	-	January 1967
University of Missouri	-	February 1967
University of Minnesota	-	December 1967
University of Illinois	-	January 1968
Kansas State University	-	January 1968
Purdue University	-	January 1968
Ohio State University	-	January 1969
University of Saskatchewan	-	January 1969
University of California	-	July 1970
Colorado State University	-	August 1972

Current Annual Level: $46,000

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1974

Special Cancer Studies Section

ALBANY MEDICAL COLLEGE OF UNION UNIVERSITY (NO1-CP-12426)

Title: Study of Histopathologic Epidemiology of Childhood Cancer

Contractor's Project Director: Dr. J. N. P. Davies

Project Officer (NCI): Robert W. Miller, M.D.

Objectives: To carry out an international study of the incidence and frequencies of childhood cancer and to determine the causes of the striking variation in incidence from one country to another.

Methods Employed: Data on all childhood cancers is currently being collected from about 20 different countries. This statistical information will be analyzed by cell type and compared with data from other centers. A histologic review of the tumor types will be carried out when specific neoplasms may provide a basis for comparison, such as ovarian tumors and lymphomas which vary greatly from country to country. The principal investigator will request blocks or sections of certain tumors for this purpose.

Major Findings: The original hypothesis, that childhood cancers vary from one country to the next, as to cell type, has proven correct. A prime example of this variation is the very low risk of Ewing's tumor among Blacks in the United States and in Africa. The results have stimulated interest in childhood cancer among the contributing centers, some of which have in consequence published reports of their findings. Also, interest at WHO was stimulated, to the extent that an expert panel was convened on childhood cancer in Geneva in December 1972 to recommend a program to improve care and research concerning childhood cancer. Twenty-one recommendations were made, and efforts are now being made to implement them locally, nationally and internationally.

Significance to NCI Program and Bio-medical Research: Marked variations in the frequency of cancer according to race and geographic origin provide opportunities to identify genetic and environmental factors in etiology. The Epidemiology Branch has been involved in epidemiologic studies of childhood cancer in the U.S. The detection and clarification of international variation in childhood cancer should increase the opportunities for generating etiologic leads. The proposed histopathologic studies should play a very integral part in illuminating these leads.

Proposed Course of Project: As data continues to be received from various cancer registries, hospitals and individual investigators from each country, tabulations and correlations will be made to elicit possible clues to origin and genetic relationships. Future analyses and further data solicitation will be made as deemed necessary to the total project.

Date Contract Initiated: June 24, 1971

Current Annual Level: $34,970

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1974

Ecology Studies Section

CASE WESTERN RESERVE UNIVERSITY (NO1-CP-43201)

Title: Mortality Experience of Children who were Inadvertently Inoculated with SV-40 very Early in Life

Contractor's Project Director: Eli Gold, M.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D.

Objectives: To determine whether the inoculation of human infants with polio vaccine containing SV-40 at birth is associated with an increased mortality due to malignant neoplasms.

Methods Employed: The mortality experience of 1,077 mainly Negro children, born in Cleveland, Ohio, between December 1959 and May 1962 and inoculated with SV-40 shortly after birth, will be followed through time. The location and name of each child are determined annually. Deaths are discovered through the use of a continuously up-dated census of Cleveland school children and from the vital records of the State of Ohio and other states to which the children have migrated. The names in the study group are compared against an alphabetic file of all childhood cancer deaths in the U.S. maintained in the Epidemiology Branch, NCI. The frequency of cancer deaths in the study group will be compared with the expected cancer mortality for children in the State of Ohio.

Major Findings: As yet there have been no cancers or other disorders in the study group attributed to SV-40. An interim report of the findings was published in Science 167: 59-60, 1970.

Significance to NCI Program and Bio-medical Research: Previous investigations by the Epidemiology Branch of school-age children who were inadvertently inoculated with SV-40 will be extended. The known importance of neonatal exposure in the causation of many viral neoplasms of animals suggests the possibility that the cancer mortality of children inoculated at birth might be different from that of children exposed at school age. The present study will extend our present knowledge in this area.

Proposed Course of Project: Since neoplasms with a latent period longer than 12 years could not be detected by the current follow-up, surveillance of the children will continue to be maintained.

Date Contract Initiated: March 9, 1964

Current Annual Level: $9,251

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1974

Ecology Studies Section

HARVARD UNIVERSITY SCHOOL OF PUBLIC HEALTH (NO1-CP-33326)

Title: Epidemiology and Etiology of Breast Cancer

Contactor's Project Director: Brian MacMahon, M.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D.

Objectives: To expedite and expand ongoing epidemiologic studies directed towards understanding the etiology of breast cancer and the identification of a preventive measure. At the present time, the studies are focusing on the hypotheses that an important determinant of a woman's lifetime breast cancer risk is the proportion of estrogens that she metabolizes to estriol during the early years of reproductive life.

Methods Employed: Comparisons are being made of urinary estrogen profiles in young women from populations with different breast cancer risks. These studies involve the following contrasts: 1) an international study, in which women from two areas of North America (Vancouver, Boston) are compared with those from three Asian centers (Nagoya, Taipai, Hong Kong); 2) a Hawaiian migrant study in which Hawaiian women of Asian descent (whose breast cancer rates are intermediate between those of North American and Asian women) are compared with Asian women in their homelands and with Hawaiian women of Caucasian descent; 3) a European study, including 3 comparisons of high-risk and low-risk populations (Denmark, Sweden, Netherlands vs. Greece, Yugoslavia; 3 Scandinavian countries vs. Finland; Oslo vs. County of Trams); and 4) a family study, involving analyses on daughters of breast cancer patients.

Other phases of the project involve an analysis of the reserve urine aliquots for androgen metabolites, a degradation study of frozen urine to calculate stability of estrogen fractions, and an experimental study on rats to clarify carcinogenic and protective potential of estrogen fractions.

Major Findings: Two geographic contrasts have been completed: women from North America are compared with those from Asia, and Hawaiian women of Asian descent as contrasted with Asian women in homelands and Hawaiian women of Caucasian descent. Both studies have shown parallels between the population's breast cancer risk and the urinary estrogen profile -- in the direction predicted by the hypothesis.

Preliminary results suggest the following 1) contrary to findings of Bulbrook, etiocholanolone levels are decreased in populations at low breast cancer risk and are inversely related to estriol proportion; 2) in experimental study on rats, administration of estriol exerts protective effect on breast cancer risk; and 3) in degradation study, urine storage for several months does not affect estrogen analyses, but storage for several years does.

Significance to NCI Program and Bio-medical research: Epidemiologic characteristics of breast cancer are consistent with hormonal determinants, which can be best evaluated by joint epidemiologic-laboratory investigations of the type planned by this contract.

Proposed Course of Project: Preliminary evidence indicates that the estrogen-fraction hypothesis should continue to be evaluated. Investigations of other hormonal determinants (androgen profiles) will be emphasized. All projects should be completed in one year.

Date Contract Initiated: June 23, 1971

Current Annual Level: $102,066

CONTRACT NARRATIVE
EPIDEMIOLOGY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

MELOY LABORATORIES, INCORPORATED (NO1-CP-43216)

Title: Viral Transformation of Cells from Persons at High Risk of Cancer

Contractor's Project Director: Mr. Donald J. Giard

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D. and William A. Blattner, M.D.

Objectives: To determine whether sensitivity to viral transformation of fibroblasts in vitro will identify persons at high risk of cancer.

Methods Employed: Studies by Todaro and his associates have revealed that skin fibroblasts from some individuals at high risk of cancer exhibit increased sensitivity to transformation by several oncogenic viruses, including SV-40. Sensitivity to transformation is evaluated by a fluorescent antibody technique which measures SV-40 induced T-antigen production in skin fibroblast culture. A new ethanol fixation technique has been developed and implemented in the last year to enhance the reliability of the test. Efforts to standardize the assay have continued with completion of testing on a large cohort of normal persons and with further testing of persons at high risk for malignancy. A refined statistical analysis of this data is underway.

Major Findings: Manuscripts are in preparation dealing with results obtained on normal individuals, and a large group of persons with leukemia. Preliminary analysis reveals significant differences between these groups. Members of a large pedigree with a long history of documented malignancy dating back to the 18th century were tested. Increased transformation rate was found in persons at highest genetic risk for malignancy. Approximately 1600 individual transformation tests were performed in the past year and approximately 300 cell lines were added to the greater than 1000 already stored and available for further study.

Significance to NCI Program and Bio-Medical Research: The Epidemiology Branch of the National Cancer Institute is concerned with studies to identify groups at high risk of cancer. This objective will be fostered by joint epidemiologic-laboratory investigations of the type planned by this contract, in collaboration with Dr. Todaro and other investigators in the Viral Oncology area of NCI. The Epidemiology Branch will continue to make this laboratory resource available to other members of the research community who have patients appropriate for study utilizing this technique. The findings should be of value not only in etiologic research, but also clinically in counseling and prevention.

Proposed Course of Project: An in-depth computer based analysis of clinical and laboratory data is being performed by clinicians and statisticians at the Epidemiology Branch to enhance the interpretation of data. Through close collaboration between clinical research and laboratory personnel, a series of

experiments aimed at improving the reproducibility and reliability of the test have been inaugurated. On completion of these experiments, testing of patient at high risk for malignancy will resume. In addition to expansion of studies on groups of patients previously studied, new areas of focus will include testing of persons with cutaneous, hereditary gastrointestinal, and multiple endocrine tumor syndromes.

Date Current Contract Initiated: October 1, 1973

Current Annual Level: $71,266.00

Ecology Studies Section

NATIONAL ACADEMY OF SCIENCES (NO1-CP-43202)

Title: Epidemiologic Studies in Etiology of Cancer in Veterans

Contractor's Project Director: Gilbert W. Beebe, Ph.D. and Seymour Jablon, M.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D. and Robert W. Miller, M.D

Objectives: To develop and conduct a broad program of epidemiologic studies in cancer among veterans.

Methods Employed: The Epidemiology Branch of the National Cancer Institute and the Follow-up Agency of the National Academy of Sciences will develop an epidemiology program designed to make efficient use of the military-veteran population, utilizing medical, demographic, and environmental observations made at entry and during military service, and the subsequent medical history of veterans ascertained through facilities of the Veterans Administration and supplemented by mortality data. The following activities were carried out during the past year: 1) a search for risk factors in Hodgkin's disease by case-control survey of 700 men under age 31 with at least 1 year of duty prior to diagnosis in a military hospital; 2) a case-control study of childhood cancer to evaluate prenatal radiation exposure, utilizing the childhood cancer mortality registry of the Epidemiology Branch, NCI, and pregnancy records available in military facilities; 3) a follow-up study of World War II veterans with certain conditions that may affect the risk of cancer, such as splenectomy, allergies, orchidopexy, nephrectomy, and alcoholism; 4) an evaluation of familial factors in cancer by means of a Twin Registry of 16,000 pairs of veteran twins, with questionnaires recently sent to living co-twins of those with any form of cancer; and 5) an extended follow-up of mortality with emphasis on respiratory cancer, among World War I veterans previously hospitalized for mustard gas poisoning.

In addition, planning activities are being made to assess the effect of adenovirus vaccine administered in 1956-58 on subsequent mortality from cancer.

Major Findings: Since this contract was initiated, reports have been published on the lack of relationship between cancer and histories of infectious mononucleosis and exposure to yellow fever vaccine containing avian leukosis virus. In the past contract year, the diagnostic index was preserved to the Korean War. No results are available on the studies outlined above.

Significance to NCI Program and Bio-medical Research: The Epidemiology Branch of the National Cancer Institute is concerned with studies to identify and

clarify etiologic factors in cancer, and these objectives can be more readily achieved by coordinating efforts with the outstanding resources and staff of the Follow-up Agency.

Proposed Course of Project: The various studies will continue into the next contract year, during which the Hodgkin's disease and mustard gas studies will be completed. New projects will be identified to clarify risk factors in cancer.

Date Contract Initiated: June 28, 1971

Current Annual Level: $99,897

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1974

Office of the Branch Chief

NATIONAL ACADEMY OF SCIENCES - NATIONAL RESEARCH COUNCIL (NO1-CP-40044)

Title: Epidemiologic research on radiogenic cancer

Contractor's Project Director: LeRoy Allen, M.D.

Project Officer (NCI): Robert W. Miller, M.D.

Objectives: To determine the frequency and nature of cancer among atomic-bomb survivors in relation to radiation dose.

Methods Employed: The Atomic Bomb Casualty Commission has had clinics in Hiroshima and Nagasaki since 1948. Cancer research will continue through a) bi-annual clinical examinations of about 20,000 survivors with a wide range of radiation dosage, b) studies of death certificates for about 100,000 people who were in either of the cities at the time of the explosions, and c) a pathology program relating to both of these study samples. In addition, under the contract, citywide tissue registries are being established under the aegis of the Hiroshima Medical Association and Nagasaki University and one-year visiting scientist programs are offered to Japanese faculty members in Departments of Epidemiology, who consult the program for about 2 months in the United States before spending the balance of the year at the Hiroshima ABCC engaged in cancer epidemiology.

Major Findings: Under the contract, none as yet.

Significance to NCI Program and Bio-medical Research: The contract will permit continuation of cancer research which has revealed that radiation in sufficient dose can induce in man several forms of leukemia and thyroid cancer. Increases in lung cancer and breast cancer are suspected, but more cases must be accumulated before the evidence is conclusive. The ABCC findings do not support claims, based on studies of low-dose diagnostic irradiation, that all forms of childhood cancer are increased by intrauterine exposure. A recent finding at ABCC that persons exposed to the bomb before 10 years of age are showing a marked increase in various forms of neoplasia at 16-33 years of age, indicates that this cohort needs special attention to determine how long elevated cancer rates persist. In addition to the information derived about radiogenic cancer, this contract opens an opportunity for greater interaction between Japanese and American cancer epidemiologists at ABCC and in general.

Date Contract Initiated: February 29, 1972

Current Annual Level: $750,000

CONTRACT NARRATIVE
EPIDEMIOLOGY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1974

WOLF RESEARCH AND DEVELOPMENT CORP. (NO1CP33309)

Title: Computer Programming Services for Mortality Studies

Contractor's Project Director: William Lake, Ph.D.

Project Officer (NCI): Mr. Thomas J. Mason

Objectives: This contract provides biomedical computing software support to a variety of mortality studies conducted by the Epidemiology Branch.

Major Findings: Wolf is providing systems analysis and computer programming support for the data processing and analysis phases of Epidemiology Branch projects. Specific activities include:

a. Computer programming to create a county population file for non-white residents;
b. Computer programming to create a county demographic file;
c. The modification of an existing sub-system to process the 1970 cancer mortality files;
d. The development of a computer system to create a national atlas index file.

Significance to Biomedical Research and the Program of the Institute: See Project Reports, Epidemiology Branch, NCI.

Proposed Course: The support represented by this contract is expected to continue through February 1974.

Date Contract Initiated: March 1, 1973.

Current Contract Level: $ 33,280

ANNUAL REPORT

CARCINOGENESIS PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE

July 1, 1973 through June 30, 1974

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I. CARCINOGENESIS PROGRAM SUMMARY REPORT

A. *INTRODUCTION*

The Carcinogenesis Program is responsible for planning, implementing, and managing the National Cancer Institute's coordinated research program on carcinogenesis by chemical and physical factors and on its prevention. In order to meet this responsibility, five coordinated program approaches have been established as follows:

1. Identification and characterization of *population groups at risk* for different cancers;

2. Identification of carcinogenic activity of selected agents by *bioassays;*

3. Development and selection of *biological models* for bioassay, for the characterization of carcinogenesis processes, and for correlation with man;

4. Identification of *processes* required for carcinogenic action of selected agents as target points for corrective measures in man; and

5. Development, application, and evaluation of *corrective measures* for man and his environment.

The Carcinogenesis Program consists of an *intramural* component devoted to laboratory research, scientific documentation and coordination, and a *collaborative* component devoted primarily to coordinated targeted research implemented through the contract mechanism. There is no programmatic distinction between the two except from an administrative standpoint. They are, in fact, designed to complement each other.

Historically, this Program developed from the Carcinogenesis Studies Branch established in March 1961 as part of the Field Studies Area, for intramural and collaborative investigations on carcinogenic factors, particularly as hazards in the human environment. With the reorganization of NCI and the establishment of the Office of the Scientific Director for Etiology in January 1966, the Carcinogenesis Program was organized under the Office of the Associate Scientific Director for Carcinogenesis (OASDC), with the task of "planning and administering a program of basic and applied research in carcinogenesis leading to the identification or definition of environmental carcinogens, and to the elucidation of carcinogenesis mechanisms." A Biology Branch and a Chemistry Branch were established to implement this program. A contract-supported collaborative program was developed since 1962 to respond to the need for extensive bioassay testing and screening of potential environmental chemical carcinogenic hazards, and for a variety of fundamental research programs on mechanisms of carcinogenesis.

The scope of the whole Carcinogenesis Program was reassessed in 1968. Following an extensive survey of the leading scientific centers in this field and a series of discussion panels convened by NCI at the request of the National Advisory Cancer Council, a Program Plan on Chemical Carcinogenesis and Prevention of Cancers was designed and approved: New directions were thus established for both intramural and collaborative activities.

At the end of FY 1968, the Carcinogenesis Program had a total personnel strength of 109 positions, all but a few totally engaged in laboratory work, often loosely related to the newly established program priorities. The contract program was at a funding level of about $5 million, with extremely limited coordination and management resources. The task of developing a highly coordinated targeted program, requiring full participation of scientific staff, development of new major program areas and establishment of managerial and technical resources, was accepted as a challenge in spite of the limited resources then available.

In 1968-1969 the intramural laboratories and offices were moved from temporary quarters to the new Building 37 on the NIH reservation.

In FY 1970 a new Experimental Pathology Branch was established besides the previously existing Biology Branch and Chemistry Branch by reassigning personnel previously in the Biology Branch and in the Office of the Associate Scientific Director. Special units were set up in the Office of the Associate Scientific Director: a Program and Data Analysis Unit, a Lung Cancer Unit, and an Epidemiologic Pathology Unit (transferred from the Demography Program). In order to provide an effective management structure to the contract-supported program, the Office of the Coordinator for Collaborative Research was established in FY 1971 and eight segments were created to manage and coordinate the expanding contract program. The Bioassay Segment was replaced in FY 1973 by two new segments (Bioassay Operations and Carcinogen Metabolism and Toxicology). The Tobacco Research Segment was transferred with its funds in October 1973 to a newly established Smoking and Health Program in the Office of the Director, DCCP; until that date, Dr. G. B. Gori, Associate Director for Program, DCCP, contributed his effort, as in previous years, as chairman of the Tobacco Research Segment and project officer on several contracts.

On December 23, 1971, the Senate and the House of Representatives of the 92nd Congress passed the National Cancer Act of 1971 (Public Law 92-218) in order to advance the national effort against cancer. Commensurate with the NCI Director's expanded program responsibilities related to the objectives of the National Cancer Act, the elevation of the Institute to bureau status and the establishment of division level components within the Institute was approved by the Secretary, DHEW, on July 27, 1972. This official organization established the former Etiology Area as the Division of Cancer Cause and Prevention (DCCP).

In FY 1972, needed organizational changes were finally made possible by the new positions provided in the increased appropriation for that fiscal year. Top priority was given to the recruitment of competent science managers fully devoted to the coordination of scientific management for the segments.

The Office of the Scientific Coordinator for Environmental Carcinogenesis, reporting directly to the Associate Director, was established. A particular task of this Office is that of developing and maintaining close liaison with other Government agencies in the field of environmental carcinogenesis, as required by the National Cancer Act of 1971. Also in FY 1972, the Registry of Experimental Cancers was transferred from the Office of the Director, NCI, to the Office of the Associate Director for Carcinogenesis. In FY 1974 a new Tumor Pathology Section was established in this Office and the Registry became a component of it.

In FY 1973 a new group of sections was established in the Experimental Pathology Branch to reflect its emphasis on the pathogenesis of cancers of the major organ systems. A Tumor Antigen Section was established in the Biology Branch. Three new branches were established at the end of FY 1973: a Carcinogen Bioassay and Program Resources Branch (from the previous Program and Data Analysis Unit), a Lung Cancer Branch (from the previous Lung Cancer Unit), and a Carcinogen Metabolism and Toxicology Branch (from the previous Carcinogen Screening Section, Experimental Pathology Branch).

In FY 1973 several offices of the Program were moved to the Landow Building and more space was thereby made available for laboratories in Building 37. Some laboratory space was rented in the Eye Research Foundation Building. The organizational expansion was an expression of the rapidly expanding needs in the Program with its increasing responsibilities in research management. Unfortunately, the availability of staff positions did not follow the pattern projected in previous years.

Already in 1969, soon after the new Carcinogenesis Plan had been approved, and at a time when a major expansion in the programs was called for, the Carcinogenesis Program was hit by the reduction in positions that affected all Institutes, and the total position strength underwent a loss of about 20%. Only at the end of FY 1971 did the position ceiling reach back to the FY 1968 level. In FY 1972 the ceiling was raised to about 145, and peaked shortly afterwards at 149. However, in FY 1973, in spite of further substantial increases in program responsibilities and budget, and in organizational commitments the personnel ceiling was cut down again to 134 positions. The ceiling remained at 135 through most of FY 1974 and was slightly raised in March 1974 to 140. This lack of consistent personnel support represents the single most serious and destructive problem in the Program and a major hindrance to its accomplishments. During these years, the Carcinogenesis staff has given invaluable cooperation to the task of substantially expanding the Carcinogenesis Program activities, both in-house and through contracts, at a time when a rapidly growing public awareness of the problems of environmental cancer hazards was giving a clear indication to the Nation of the increasing needs in this field.

In the last 18 months, in spite of the critical shortage of positions, thanks to the untiring efforts and cooperation of the staff, several major contract programs were initiated. However, major activities had to be curtailed due to the loss of key personnel that could not be replaced, and to the lack of sufficient support personnel in several parts of the program.

The Epidemiologic Pathology Unit was closed down after the retirement of two of its three full-time professionals, thus depriving the Carcinogenesis Program of a most important link with the field of human cancer epidemiology.

The Chief of the Experimental Pathology Branch decided to leave this position as well as that of Director of the Biological Models Segment. It has not been possible to fill these vacancies in the absence of proper support personnel. The sections on Endocrine Carcinogenesis and Digestive Tract Carcinogenesis in the Experimental Pathology Branch, which had been established as an in-house basis of expertise for the development of a collaborative program in these two most important areas of carcinogenesis research with high human relevance, could not be adequately staffed. Therefore the endocrine program is being transferred to the Carcinogenesis area of the NCI's Frederick Cancer Research Center, while a digestive tract carcinogenesis program could not be developed under the present circumstances.

Environmental carcinogenesis activities, which were planned to extend considerably in view of government-wide commitments in this field, could not be supported by new personnel: They remain mostly at the level of liaison with other federal agencies.

The new Tumor Pathology Section, with its critical role of developing and monitoring pathology standards throughout the Carcinogenesis Program has not yet been provided with much needed additional staff. Finally, the Carcinogen Bioassay and Program Resources Branch continues to bear a major program responsibility with an extremely exiguous staff of three professionals. All other parts of the Program are already at a minimum level of staff and any personnel losses result in curtailing major program efforts. The recent assignment of five new positions will not go very far in restoring the losses of an already overburdened staff.

During FY 1973 a major development of the Carcinogenesis Program has been the establishment of a large operation at the NCI's Frederick Cancer Research Center. The Carcinogenesis Program has undertaken to support about 40% of the Center's activities including long-term animal bioassays, preparative and analytical chemistry resources, in vitro carcinogenesis methods, studies on intestinal bacterial flora in carcinogen metabolism related to colon cancer and carcinogen safety handling. A program of carcinogenesis research has been initiated in the current fiscal year with emphasis on endocrine carcinogenesis. Plans have been made for a major expansion of the Carcinogenesis Program at the Frederick Cancer Research Center through the recruitment of highly qualified scientists in experimental pathology, cell biology, and molecular biology, committed to the field of carcinogenesis research. Dr. R. R. Bates (formerly Chief of the Experimental Pathology Branch) accepted the position of Scientific Coordinator for Carcinogenesis at the Frederick Cancer Research Center.

Members of the staff have participated in the development of international cooperative programs, related to carcinogenesis. A panel on mutagenesis and carcinogenesis was established and held a meeting in conjunction with the National Institute of Environmental Health Sciences for the US-Japan Cooperative Medical Science Program. The US-USSR program on tumor cell genetics

has held four meetings. This program is concerned primarily with mammalian somatic cell genetics related to neoplasia. Two areas will be developed--mutagenesis and cell hybridization.

An ad hoc Committee on Testing for Environmental Chemical Carcinogens, established by the Director, NCI, completed a review of the activities of agencies of the U.S. Government in this field. A final report was submitted to the Director, NCI, on August 31, 1973. The Committee recognized that there is no legal requirement for systemic testing for carcinogenicity of chemicals present in or being introduced into the environment. The current NCI Carcinogenesis bioassay program was found to be the closest approximation to this need. It was recommended that (1) consideration should be given to the establishment of a Federal Council on Environmental Carcinogenic Hazards, (2) NCI should develop a comprehensive national program for identification of carcinogenic chemical hazards in the environment, with a view to their elimination or control, (3) the cost of the program should be borne jointly by the various agencies and organizations involved, (4) resources required should be provided without delay, and (5) a unit within the NCI should be charged with these responsibilities. Several of the items recommended are already being implemented by the Carcinogenesis Program. Additional financial and personnel resources would be required to accomplish the full program scope recommended by this Committee.

The Carcinogenesis Program has developed an articulated structure highly responsive to scientific program needs and priorities and at the same time highly qualified for scientific competence and in-house research accomplishments. All but a few professional members of the staff share a direct interest and involvement in the total program, participating in varying degrees to both their intramural research activities and the coordination of the collaborative program. Their high degree of scientific productivity--attested by an imposing contribution to the scientific literature, and by their standing in the scientific community--was coupled with an increasing dedication to the development of effective research teams throughout the country, supported by the contract program.

The crisis due to the drastic cutback in personnel in FY 1973, which has threatened the very existence of a national Carcinogenesis Program has not been resolved. This situation occurs at a time when new high priority programs are developing as a direct response to pressing national needs in environmental and occupational carcinogenesis in the attack against lung cancer, cancers of the digestive tract and endocrine-related cancers, as well as in the most urgent area of the development of short-term in vitro methods for chemical carcinogenesis tests and evaluations--a field where major breakthroughs have been accomplished by this Program and have generated great expectations in the scientific community, in the federal regulatory agencies, in industry, in the labor unions, and in the consumer protection movement.

The organization, the scientific activities, and the progress of the Carcinogenesis Program are reported in the pages that follow.

B. *ORGANIZATION*

The organization of the Carcinogenesis Program is presently divided into two major components: intramural program and collaborative program. The former is articulated in the Office of the Associate Director for Carcinogenesis and in six branches, each with several sections and units. The latter is articulated into eight segments. Each segment is directed by a senior staff member, assisted by a manager; other members of the staff serve as project officers on individual contracts. Each segment is assisted by a segment advisory group with members from outside NCI. Three other advisory groups provide program-wide scientific consultation and review.

1. INTRAMURAL PROGRAM

The Office of the Associate Director for Carcinogenesis (U. Saffiotti, M.D., Associate Director; J. A. Cooper II, Ph.D., Deputy Associate Director):

- Office of the Scientific Coordinator for Environmental Carcinogenesis (H. Kraybill, Ph.D., Scientific Coordinator)
- Office of the Scientific Coordinator for Carcinogenesis at the NCI's Frederick Cancer Research Center) (R. R. Bates, M.D., Scientific Coordinator)
- Tumor Pathology Section (R. A. Squire, D.V.M., Head; M. Barrett, M.D., C. Lingeman, M.D. (T. Dunn, M.D., H. L. Stewart, M.D., Consultants)

The Biology Branch (H. J. Rapp, Sc.D., Chief; T. Borsos, Sc.D., Associate Chief):

- Cellular Immunity Section (B. Zbar, M.D., Head; R. Bast, M.D., R. Harmel, M.D.)
- Cytogenetics and Cytology Section (J. A. DiPaolo, Ph.D.; Head, P. Donovan, C. Evans, M.D., B. Myhr, Ph.D., N. Popescu, Ph.D.)
- Immunochemistry Section (T. Borsos, Sc.D., Head; B. Hooks, Ph.D., S. Ohanian, Ph.D., M. Segerling[1])
- Tumor Antigen Section (E. Leonard, M.D., Head; D. Boetcher, M.D., M. Meltzer, M.D., H. Smith, M.D.)

The Carcinogen Bioassay and Program Resources Branch (N. P. Page, D.V.M., Chief; T. Cameron, D. V. M., T. Kuch[1], M. Linhart, S. Siegel, Ph.D.)

The Carcinogen Metabolism and Toxicology Branch (E. K. Weisburger, Ph.D., Chief; T. Glinsukon, Ph.D.[1]):

- Metabolic Studies Section (E. K. Weisburger, Ph.D., Acting Head; P. Grantham, J. Idoine, L. Poirier, Ph.D., J. M. Ward, D.V.M., Ph.D.)
- Analytical Chemistry Unit (L. K. Keefer, Ph.D., Acting Head; P. Roller, Ph.D.)
- Bacterial Metabolism Unit (S. Silverman, Ph.D., Acting Head)

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[1]Guest Worker

The Chemistry Branch (H. V. Gelboin, Ph.D., Chief):

Cell Growth Regulation Section (J. P. Bader, Ph.D., Head; R. Harris, Ph.D., D. Hatfield, Ph.D.)
Molecular Carcinogenesis Section (H. V. Gelboin, Ph.D., Acting Head; K. Nishinaga, Ph.D.[1], J. Selkirk, Ph.D., J. Whitlock, M.D., F. Wiebel, M.D.)
Nucleic Acids Section (C. W. Dingman, M.D., Head; R. Day, M.D., T. Kakefuda, M.D., T. Kakunaga, Ph.D.)
Protein Section (A. C. Peacock, Ph.D., Head; S. Bunting, M. Green, Ph.D., J. V. Pastewka)

The Experimental Pathology Branch (J. M. Rice, Ph.D., Acting Chief):

Digestive System Carcinogenesis Section (J. M. Rice, Ph.D., Acting Head; M. Lieberman, M.D., R. Yamamoto, Sc.D.)
Endocrine Carcinogenesis Section (D. Janss, Ph.D., Acting Head; B. Chen, Ph.D., W. Edwards, Ph.D.[1])
In Vitro Pathogenesis Section (S. Yuspa, M.D., Head; P. Dermer, M.D., H. Hennings, Ph.D., D. Morgan)
Perinatal Carcinogenesis Section (J. M. Rice, Ph.D., Head; S. Joshi, Ph.D.)
Histopathology Unit (D. G. Kaufman, M.D., Acting Head)
Ultrastructure Unit (C. C. Harris, M.D., Acting Head)

The Lung-Cancer Branch (M. B. Sporn, M.D., Chief; J. Smith):

Differentiation Control Section (L. De Luca, Ph.D., Head; R. Barr, Ph.D.[1], S. De Luca, Ph.D.[1])
Lung Cancer Pathogenesis Section (M. Sporn, M.D., Head; G. Clamon, M.D., A. Frank, M.D., V. Genta, M.D. , C. C. Harris, M.D., D. Kaufman, M.D.)

2. COLLABORATIVE PROGRAM

The Office of the Associate Director for Carcinogenesis (U. Saffiotti, M.D., Associate Director; J. A. Cooper II, Ph.D., Deputy Associate Director; A. H. Heim, Ph.D., Coordinator for Collaborative Research)

Bioassay Operations Segment (N. P. Page, D.V.M., Director; J. Sontag, Ph.D., Acting Manager)

Biological Models Segment (R. Pledger, Ph.D., Acting Director; R. Pledger, Ph.D., Manager)

Biology and Immunology Segment (H. J. Rapp, Sc.D., Director; V. Dunkel, Ph.D., Manager)

Carcinogen Metabolism and Toxicology Segment (E. K. Weisburger, Ph.D., Director; J. Sontag, Ph.D., Manager)

[1]Guest Worker

Chemistry and Molecular Carcinogenesis Segment (A. C. Peacock, Ph.D., Director; A. E. Kaplan, Manager)

Colon Cancer Segment (S. Silverman, Ph.D., Acting Director; A. E. Kaplan, Acting Manager)

Information and Resources Segment (J. A. Cooper II, Ph.D., Director; M. D. Litwack, Ph.D., Manager)

Lung Cancer Segment (M. B. Sporn, M.D., Director; C. Smith, Ph.D., Manager)

3. ADVISORY GROUPS AND CONSULTANTS

The *Carcinogenesis Advisory Panel* provides overall discussion and advice to the Carcinogenesis Program staff on the development of the program and its priorities in the context of future activities of the National Cancer Institute.

The *Carcinogenesis Contract Program Management Group* provides review for need, priority, and relevance for the whole program.

The *Carcinogenesis Intramural Program Group* provides a coordinating and planning function at the senior staff level. The entire professional staff is now essentially participating in the planning and monitoring of the overall program, providing a vital correlation between intramural and collaborative research programs.

The *Segment Advisory Groups* have been established to provide advice on program needs and priorities as well as technical review of contract proposals. They all include several members from outside NCI and they do not receive contract support from the segment on which they are an advisor.

The group of individual advisors to the Carcinogenesis Program at the NCI's Frederick Cancer Research Center has been established to review the quality of the ongoing effort and to provide a stimulus for the development of important new project areas.

In compilance with departmental guidelines, plans are being made to reduce the number of advisory committees.

Close liaison continues to be maintained with the Cancer Cause and Prevention Program Advisory Committee at the Division level, and it is hoped that a more effective liaison will be established with the National Cancer Advisory Board.

a. *Carcinogenesis Advisory Panel*

Dr. John Baldeschwieler, Chairman, Department of Chemistry, California Institute of Technology, Pasadena, California

Dr. John Burns, Vice President for Research, Hoffmann-La Roche, Inc., Nutley, New Jersey

Dr. Emmanuel Farber, Professor of Pathology, Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania

Dr. Theodore Hauschka, Director, Cancer Research (Biology), Roswell Park Memorial Institute, Buffalo, New York

Dr. Ruth Kirchstein, Deputy Associate Commissioner for Science, Food and Drug Administration, Rockville, Maryland

Dr. Paul Kotin, Vice-President, Health Sciences Center, Temple University School of Medicine, Philadelphia, Pennsylvania

Dr. Peter Magee, Professor of Biochemistry, Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, England

Dr. James A. Miller, Professor of Oncology, McArdle Memorial Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin

Dr. Norton Nelson, Director, Institute of Environmental Medicine, New York University Medical Center, New York, New York

Dr. Jesse Steinfeld, Chairman, Department of Oncology, Mayo Clinic and Foundation, Rochester, Minnesota

Dr. Arthur Upton, Dean, School of Basic Health Sciences, State University of New York, Stony Brook, New York

Dr. Gerald Wogan, Professor of Food Toxicology, Massachusetts Institute of Technology, Cambridge, Massachusetts

b. *Carcinogenesis Contract Program Management Group*

Dr. Umberto Saffiotti, Associate Director for Carcinogenesis, DCCP, NCI, *Chairman*

Dr. Allen H. Heim, Coordinator for Collaborative Research, Carcinogenesis, DCCP, NCI, *Executive Secretary*

Dr. Richard R. Bates, Scientific Coordinator for Carcinogenesis, NCI's Frederick Cancer Research Center, OADC, DCCP, NCI

Dr. John A. Cooper II, Director, Information and Resources Segment

Dr. Thaddeus Domanski, Program Director for Carcinogenesis, Division of Cancer Research Resources and Centers, NCI

Dr. Harry Gelboin, Chief, Chemistry Branch, Carcinogenesis, DCCP, NCI

Dr. Herman Kraybill, Scientific Coordinator for Environmental Carcinogenesis, OADC, DCCP, NCI

Dr. Norbert P. Page, Director, Bioassay Operations Segment

Dr. Andrew C. Peacock, Director, Chemistry and Molecular Carcinogenesis Segment

Dr. Richard Pledger, Acting Director, Biological Models Segment

Dr. Herbert J. Rapp, Director, Biology and Immunology Segment

Dr. Jerry M. Rice, Acting Chief, Experimental Pathology Branch

Dr. Michael B. Sporn, Director, Lung Cancer Segment

Dr. Elizabeth K. Weisburger, Director, Carcinogen Metabolism and Toxicology Segment

c. *Carcinogenesis Intramural Program Group*

Dr. Umberto Saffiotti, Associate Director for Carcinogenesis, DCCP, NCI, *Chairman*

Mr. Damian Crane, Administrative Officer for Carcinogenesis, DCCP, NCI, *Executive Secretary*

Dr. Richard R. Bates, Scientific Coordinator for Carcinogenesis, NCI's Frederick Cancer Research Center, OADC, DCCP, NCI

Dr. John A. Cooper II, Deputy Associate Director for Carcinogenesis, DCCP, NCI

Dr. Harry V. Gelboin, Chief, Chemistry Branch, Carcinogenesis, DCCP, NCI

Dr. Allen H. Heim, Coordinator for Collaborative Research, OADC, DCCP, NCI

Dr. Herman Kraybill, Scientific Coordinator for Environmental Carcinogenesis, OADC, DCCP, NCI

Dr. Norbert P. Page, Chief, Carcinogen Bioassay and Program Resources Branch, Carcinogenesis, DCCP, NCI

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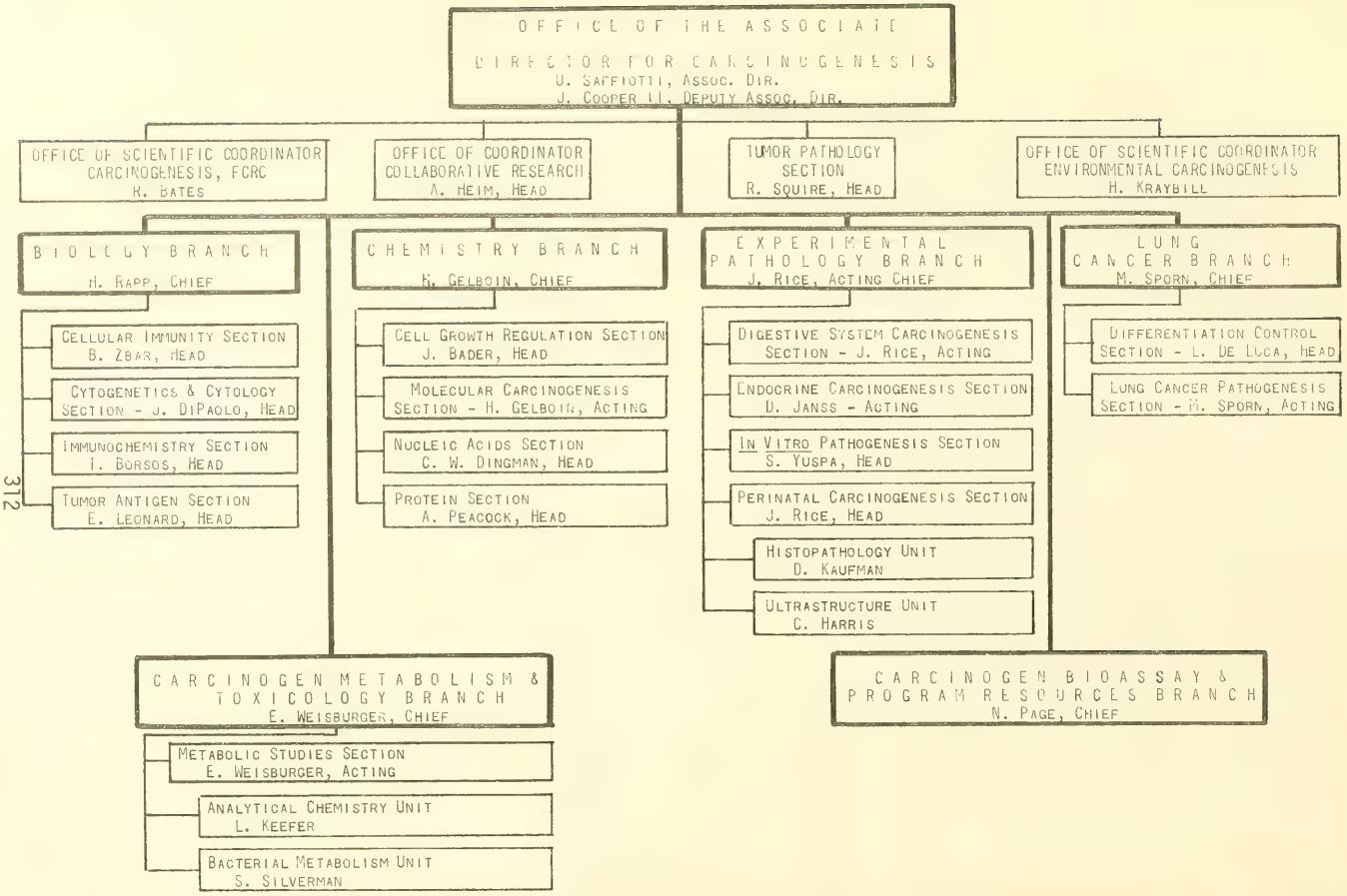
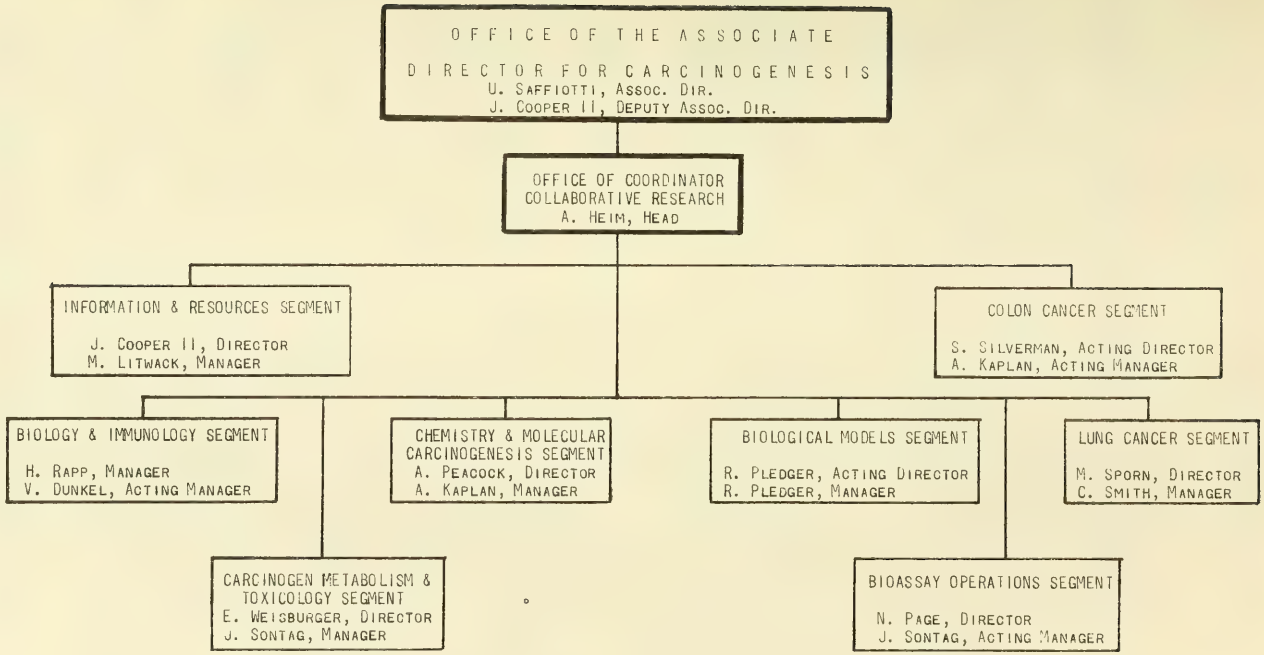


INTRAMURAL ORGANIZATION



COLLABORATIVE RESEARCH ORGANIZATION

C. *MANAGEMENT ACTIVITIES*

(Report from the Office of the Coordinator for Collaborative Research)

The philosophy that a science-based management system is essential for effective management of a complex contract-supported collaborative program remains fundamental to the way in which management activities are conducted. This is reflected in the continued use of program segments staffed by (1) intramural scientists actively engaged in research who provide scientific direction and technical expertise, (2) scientist-administrators who provide management expertise, and (3) outside consultants who provide technical expertise, advice, and review as a peer group. Each segment continued to have responsibility for developing its own specific program in keeping with the fundamental objectives of the segment but within the framework of the overall Carcinogenesis Program. As a basis for the implementation of its operational plans, each segment receives a preliminary budget apportionment from the Carcinogenesis budget. While this is an essential factor to good planning and program management, it also represents a realistic limiting factor requiring some difficult priority choices between continuing or expanding existing programs and initiating new ones. When new ones are initiated, requests for proposals (RFPs) are formulated at the segment level and proposals are reviewed by the segment for technical merit and relevance to the segment program. Recommendations for support of a particular research program and establishment of a contract are made by the segment to the Carcinogenesis Contract Program Management Group (CCPMG).

The CCPMG is an advisory group which includes all segment directors and serves the following functions. It provides review of proposals for need, priority, and relevance within the total Carcinogenesis Program; it functions in an advisory capacity to the Associate Director for Carcinogenesis; and it reviews RFPs before they are advertised for competitive proposals. This last function is designed to prevent the initiation of inadequately structured RFPs and more importantly, to prevent the initiation of a request for work which may be considered to be of low relevance and priority to the overall program. Membership of the CCPMG is as follows: the Associate Director for Carcinogenesis (Chairman); the Coordinator for Collaborative Research; the Scientific Coordinator for Environmental Carcinogenesis; the Acting Scientific Coordinator for Carcinogenesis at the NCI's Frederick Cancer Research Center; all of the segment directors and branch chiefs, and the Program Director for Carcinogenesis, Division of Cancer Research Resources and Centers, (to provide liaison with the grants program). Segment managers, contract specialists, the administrative officer, and appropriate project officers attend the meetings as observers and resource staff. The membership and resource staff of this senior advisory group represents all of the major elements in Carcinogenesis: the Office of the Associate Director for Carcinogenesis, intramural scientific staff, collaborative program scientific and management staff, contract specialists, and Carcinogenesis' administrative staff, as well as the representative from the Division of Cancer Research Resources and Centers.

Overall review and advice is provided by the Carcinogenesis Advisory Panel whose members are leading scientists in carcinogenesis and related fields from the scientific community outside of NCI. This Panel evaluated the

current status of carcinogenesis research and advises the Associate Director on overall program direction to enable him to respond to needs within the area of carcinogenesis.

The development and review procedures are currently being revised to meet new guidelines of committee management. However, peer review will continue to be emphasized and the procedures will not be different in principle from those used during this fiscal year and illustrated schematically below.

Responsibility for overall collaborative program management rests with the Coordinator for Collaborative Research who is on the staff of the Associate Director for Carcinogenesis. Responsibility and legal authority for the administrative aspects of contracting rests with the NCI Contract Officer and with contract negotiators working with the Carcinogenesis Program staff. Although Program staff and contract staff each has specific areas of responsibility, the two actually work closely together in the conduct of the collaborative program.

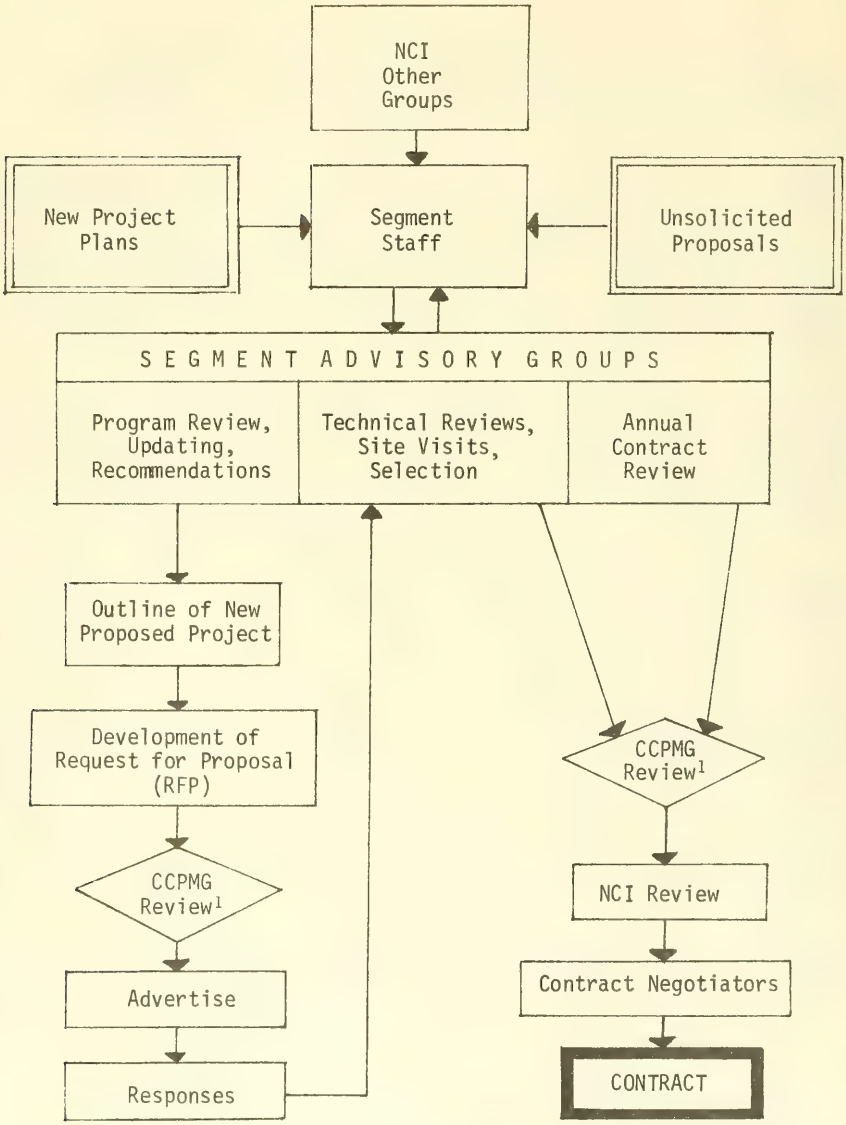
During the past year each segment has had a series of meeting usually between staff and consultants but at times including contractors for the purpose of program planning and proposal review. These meetings were scheduled as dictated by the workload of the segment and resulted in each one meeting about three times during the year. Visits to investigators were carried out as needed throughout the year. The CCPMG met regularly each month. The following activities were carried out in support of the Collaborative Program: (1) 25 segment advisory group meetings; (2) two Carcinogenesis Advisory Panel meetings; (3) approximately 100 visits to contractors; and (4) the Second Annual Carcinogenesis Collaborative Conference.

The Second Annual Carcinogenesis Collaborative Conference was held in November 1973 in San Antonio, Texas. It was attended by approximately 350 participants, including staff, advisors, contractors and members of other government agencies. The Conference provided an opportunity for participants to become acquainted with the overall program and its goals; it provided participants an opportunity to become acquainted with specific segment activities and it also provided them with an opportunity to participate in several symposia designed to provide a forum for the presentation and discussion of the latest data in various areas of research critical to the success of the Carcinogenesis Program. A third conference is being planned for early 1975.

Management responsibilities continued to increase with the program growing to approximately 170 contracts totalling about $31,000,000 by the end of Fiscal Year 1974. Approximately 25 RFPs were issued and 75 unsolicited proposals were reviewed. The activities of the Tobacco Research Segment have been transferred from Carcinogenesis to the Smoking and Health Program.

Basically the segment system serves the needs of the Carcinogenesis Program quite well in the manner in which it provides for interaction and utilization of talents of each type of professional involved: the scientist-administrator, the bench scientist, and the consultant. However, it may help the reader of this report who has an interest in management to gain a fuller appreciation of the accomplishments of the Carcinogenesis Program staff by being made aware of some of the ways in which the system is stressed; an important factor not

CARCINOGENESIS CONTRACT DEVELOPMENT AND REVIEW PROCESS



[1]CCPMG - Carcinogenesis Contract Program Management Group

reflected in a simple recounting of numbers of contracts and contract actions. First, it must be noted that within the segments, the manager is the only staff member whose responsibilities pertain exclusively to the conduct of the collaborative program. Intramural staff members have research or administrative responsibilities and consultants have primary responsibilities to their own organizations. Staffing limitations imposed on the NCI are reflected at program levels and as a consequence in Carcinogenesis there are six managers to manage eight segments with a combined activity, as stated above, of about 170 contracts totalling $31,000,000. This provides one with some concept of the workload each must assume in meeting responsibilities for planning, reviewing and monitoring the research projects. In addition there are no junior professionals for assistance, and in fact, each manager does not have his own secretary. There are two secretaries and a trainee available to the six managers; a major factor considering the requirements for documentation, preparation of review background materials, meeting arrangements, correspondence, travel, etc. all within the context of one or another deadline.

Again, in the interest of reviewing and understanding the management activities a brief consideration of some of the stresses affecting them is appropriate. An underlying factor is a geniune concern of responsible NCI, NIH and DHEW staff for the fair and proper management of large sums of public funds, in this instance within the context of a targeted Collaborative research effort. As a consequence of the magnitude of the effort, responsibilities and authority have had to be delegated to various components with expertise in areas such as contracting, committee management, planning etc. Each of these areas of responsibility issues guidelines to the operating program level designed to be responsive to public needs as they perceive them. In short, what this illustrates is that whereas government is often accused of being unresponsive, in fact, many of the stresses on program management result from being concerned and too responsive to various interest groups. Examples of how this effort to be responsive affects management activities at the operating level are as follows. Concern has been expressed that before a contract is awarded every effort be made to advertise as widely as possible the need for research and for proposals in specific areas and for appropriate review of the relevance and priority of the concept as well as sound technical reviews of the proposals. To truly respond to this concern it is essential that competition be stressed and that consultants be utilized to strengthen and broaden the expertise available for technical reviews and for program planning. These are reasonable requirements and are of great benefit to the program staff and to the academic and commercial scientific community. Implementation of this policy at the operating level is complicated, however, by trying to be responsive to several other concerns. One is a concern about the time involved for initiation and review of a project; it should be as short as possible. On the other hand, attempts are made to increase competition by using the NIH Guide for Grants and Contracts to advertise requests for proposals to the academic community and using the Commerce Business Daily to advertise to the commercial community. After a project is conceived or proposals are received, it is necessary to have reviews by two different groups - one for relevance and priority and one for the scientific merit. Emphasis, as mentioned, is on use of peer review. This procedure of widely advertising and carrying out adequate peer review is necessarily time consuming and consequently necessarily counter to rapid initiation of a project.

Time, however, is possibly the least complicating factor in the review process. Establishment of a workable peer review system within the guidelines is far more challanging. This results from a concern that too many committees are involved in these procedures. Again, a valid concern but one which must be met with a reasonable balance between good peer review and the number of scientists involved in that process. This balance would be readily attainable except for additional concerns that the public be kept appraised of committee actions and indeed be invited to participate to a degree by holding a portion of committee meetings as a public forum. This, in turn, has resulted in a requirement that any group making a recommendation which has one non-Federal member be chartered as a committee, advertise its meetings in the Federal Register and publicize its actions. Again, of itself, a reasonable requirement but it takes no imagination at all to recognize the fact that it is virtually impossible to apply this requirement within the context of obtaining reasonably rapid and meaningful peer review of research proposals, utilize non-Federal experts and at the same time reduce the number of committees. There are several alternatives that occur but are not readily applied because of attempts to respond to other expressed concerns. The first is to reduce the use of non-Federal consultants and have staff assume the major role. This, however, is counter to current efforts to limit staff growth and to avoid making contract supported research an extension of intramural research. There are additional efforts, in fact, to have the staff assume even less responsibility for review and to have review groups contain a majority of non-staff members. A second alternative is to utilize consultants on an ad hoc basis but this precludes their making joint recommendations and could weaken the peer review process. There are other factors affecting the review specifically but these should suffice to illustrate some of the difficulties presented to management staff.

There are other stresses on the system such as: 1) attempting to have as much competition as possible but also to provide a channel for receipt and review of unsolicited proposals; 2) operating throughout most of the fiscal year without a defined budget, meeting a May 1st deadline for completion of all requests for contract actions and still utilize funds wisely; 3) develop programs within the context of the National Cancer Plan which is itself undergoing a revision process; and 4) conduct site visits with staff and at least two consultants, for each new contractor receiving an award in excess of $100,000 before the award is made, also for each on-going contract at least one time every three years; and this while trying to reduce travel, speed up the review process and reduce the number of committees. The last point is significant because the use of non-Federal consultants even for site visits constitutes the formation of a committee which requires chartering etc. if that site visit team issues a group recommendation.

In summary, the above guidelines are only illustrative of those under which the management staffs have been operating and in most instances, each by itself is reasonable. However, together they run counter to each other at times and result in stressing our management system appreciably and certainly challange it to be effective. We must continually strive to find a proper balance between responding to a variety of public concerns and being able to fulfill our responsibilities for sound and effective management of programs supported by public funds and designed to promote the common good.

Finally, it is a pleasure to acknowledge the excellent efforts of the staff of the Cancer Cause and Prevention Contracts Section. Their continual support and guidance has contributed immeasurably to the progress made by the Carcinogenesis Program.

D. *PROGRAM PLANS*

The overall plan for the Carcinogenesis Program was laid down in 1968 and has served as a solid basis for the implementation of new efforts, as a guide for the expansion of the Program and as a reference for its monitoring. Particular emphasis is now given to the development of special programs on the main organ-specific types of cancers, as seen in man. The first of these to be implemented was the Special Lung Cancer Program, developed in FY 1969, partly by the Lung Cancer Task Force and by the Tobacco Working Group. Its general structure was patterned on that of the general Carcinogenesis Plan. An intramural Lung Cancer Unit was established to serve as a research focus in this effort and to provide strong correlation between staff and contractors.

A special plan has been developed on the etiology of colon cancer, through an ad hoc group chaired by Dr. John Berg, with the collaboration of several staff members in close liaison with the National Task Force on Colon Cancer. A contract program has been established in this important area. Two other special programs have been initiated by the Experimental Pathology Branch and the Biological Models Segment on cancer of the prostate and on cancer of the pancreas, again in close liaison with national efforts in these fields.

In response to the planning needs for the development of the new National Cancer Program, the Carcinogenesis staff has outlined a five-year plan and developed a number of supporting documents, including construction plans and plans for the utilization of research facilities at the Frederick Cancer Research Center.

A summary of the general Carcinogenesis Plan is given below.

Program Plan on Chemical Carcinogenesis and Prevention of Cancers

The plan was developed in 1968 through an extensive series of planning sessions conducted by Dr. Carl G. Baker, then Scientific Director for Etiology, Dr. Umberto Saffiotti, Associate Scientific Director for Carcinogenesis, and Mr. Louis M. Carrese, with the participation of Dr. Abraham Cantarow, and Mr. Richard A. Terselic. The Report of the Discussion Groups on Chemical Carcinogenesis, coordinated by Dr. Cantarow for the National Advisory Cancer Council, served as an excellent basis for outlining the research needs in the field of carcinogenesis. The convergence technique was used to identify the major objectives and the linear array of subsequent phases leading to their implementation. An outline of the program plan is attached (see Chart) and its main phases are discussed below. The program is articulated in a number of segments. The first segment deals with (a) the epidemiologic identification of population groups showing different risks to different types of cancer and (b) to the characterization of such individuals as to their environmental factors and their biological and functional parameters. The program then develops the following distinct approaches:

(1) identification of carcinogenic activity of selected chemicals by bioassay,

(2) identification, development, and selection of biological models for carcinogenesis bioassays as well as for the characterization of carcinogenesis processes, and

(3) identification of processes required for the carcinogenic action of selected agents as target points for corrective measures.

The first approach requires an initial phase devoted to the identification and selection of chemical agents to be entered in the bioassay systems. The development and implementation of bioassay procedures will require a great deal of accurate standardization so that selected chemical agents will be tested through a battery of precisely defined and reproducible bioassay systems, each well characterized for its sensitivity to specific carcinogenic effects.

After appropriate monitoring for relevance and for program needs, the results of the first phases of the program will lead to the development of corrective measures based, on one hand, on modifications of the environment and, on the other hand, based on modifications of individual target points in the process of carcinogenesis, identified by previous studies. These target points can be visualized as taking place at any one of the several steps required for the process of carcinogenesis to be completed. They include the following: (a) penetration of the chemical agent into the whole organism; (b) transport, retention and elimination of the chemicals in tissues (molecular logistics); (c) metabolic pathways to proximate carcinogen; (d) cell and tissue factors required for the previous steps; (e) penetration into target cells and interaction with cell constituents; (f) neoplastic transformation and conditioning factors; and (g) growth regulation of transformed cells.

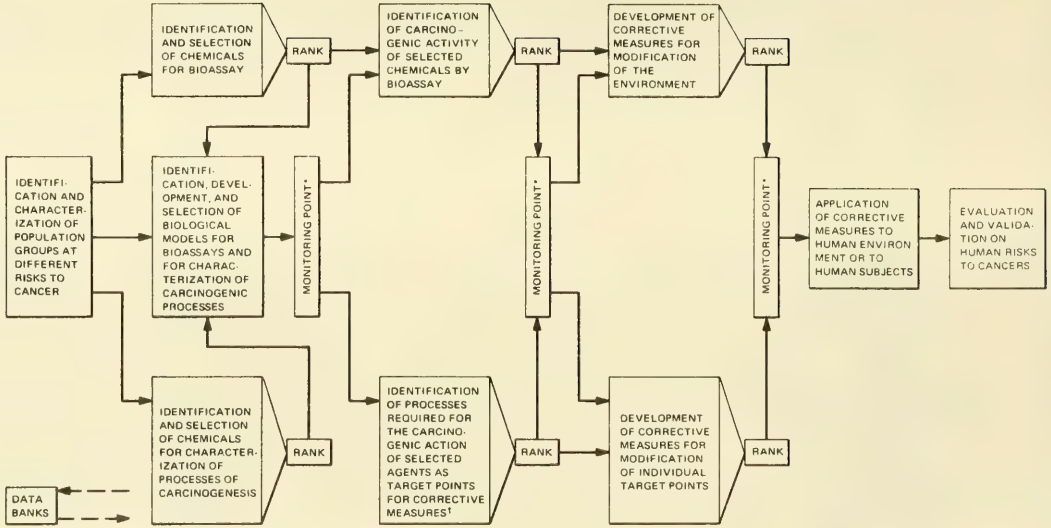
The results thus obtained will be in turn appropriately monitored for relevance; the program then considers the development of corrective measures and ultimately suggests application of such corrective measures through the human environment or through the human subject. The last phase of the program deals with the evaluation and validation of the results of such corrective measures in the human situation and is expected to lead to the recognition of decreased cancer risks and in the populations originally studied in Phase I. Data banks will be organized for each of the major phases of the program and they will be a necessary source of information to be used for the monitoring of the whole program.

The program is aimed at a comprehensive attack to the problem of chemical carcinogenesis. It recognizes the need for very extensive bioassay efforts of an order of magnitude much greater than that presently available, but also recognizes that the accumulation of information on the carcinogenicity of chemicals in animal systems will not provide a direct extrapolation to the hazards in man. Moreover, it is predictable that a large number of chemical carcinogens will be difficult to remove and, therefore, will remain in the environment of man for a long time to come even after being identified (e.g. combustion products, cigarette smoke, natural food contaminants such as mycotoxins, metals and other air pollutants). It is, therefore, imperative that an effort be made not only to identify and possibly remove an increasing number of carcinogens from the environment, but also, and concurrently, to

identify steps in their mode of penetration into the organisms and the target tissues and in their critical interaction with cell constituents so that these steps could be exploited as target points for inhibitory or protective measures. The considerable development of refined technical methods presently available for the identification of these critical steps in carcinogenesis by studies in analytical chemistry, biochemistry, cell biology, molecular biology, and immunology can now be brought to bear on our ability to develop useful, protective measures against the ultimate effects of the carcinogenic process. The identification of critical parameters required for the carcinogenic action of certain groups of chemicals, obtained in animal studies, also represents the only direct link that can be applied to the study of the susceptibility of man to comparable exposure conditions.

For example, the establishment of a strong correlation between the enzymatic activation of certain chemicals to the proximate carcinogen by means of given groups of enzymes, such as the microsomal aryl hydroxylase for polynuclear hydrocarbons may lead to the identification of groups of individuals as being highly susceptible to certain specific carcinogenic exposures, pharmacologic inhibition of some of these enzyme systems may lead to a marked decrease of the cancer risk for such individuals. As other examples, analogous approaches can be made at the level of the control of protein synthesis in differentiation-dependent carcinogenic mechanisms (e.g. hormone-dependent tumors and vitamin-A dependent tumors) and at the level of the immunological control of the growth of transformed cells. A broad front of attack can thus be developed against the sequence of events required for the carcinogenic process to lead to the establishment of progressively growing invasive tumors.

The effective development of such studies depends in part on the availability of appropriate animal models, capable of reproducing tumor types analogous to those observed in man. The development of appropriate biological models is particularly important for the establishment of selectively sensitive bioassay systems. An important phase of the program consists in the selection and standardization of bioassay systems that could be reproduced in several laboratories under strictly controlled standardized conditions. This is an essential requirement if we want to collect and analyse large series of data on carcinogenesis tests. For this purpose, a detailed plan is being worked out for a data retrieval and analysis system for carcinogenesis studies. The present overall Program Plan in Chemical Carcinogenesis and Prevention of Cancers constitutes the basis for the development of detailed operational plans aimed at the implementation of each of its phases.


IDENTIFICATION AND SELECTION OF CHEMICALS FOR BIOASSAY
RANK
IDENTIFICATION OF CARCINO-GENIC ACTIVITY OF SELECTED CHEMICALS BY BIOASSAY
RANK
DEVELOPMENT OF CORRECTIVE MEASURES FOR MODIFICATION OF THE ENVIRONMENT
RANK
IDENTIFI-CATION AND CHARACTER-IZATION OF POPULATION GROUPS AT DIFFERENT RISKS TO CANCER
IDENTIFI-CATION, DEVEL-OPMENT, AND SELECTION OF BIOLOGICAL MODELS FOR BIOASSAYS AND FOR CHARAC-TERIZATION OF CARCINOGENIC PROCESSES
MONITORING POINT*
MONITORING POINT*
MONITORING POINT*
APPLICATION OF CORRECTIVE MEASURES TO HUMAN ENVIRON MENT OR TO HUMAN SUBJECTS
EVALUATION AND VALIDA-TION ON HUMAN RISKS TO CANCERS
IDENTIFICATION AND SELECTION OF CHEMICALS FOR CHARACTER-IZATION OF PROCESSES OF CARCINOGENESIS
RANK
IDENTIFICATION OF PROCESSES REQUIRED FOR THE CARCINO-GENIC ACTION OF SELECTED AGENTS AS TARGET POINTS FOR CORRECTIVE MEASURES†
RANK
DEVELOPMENT OF CORRECTIVE MEASURES FOR MODIFICATION OF INDIVIDUAL TARGET POINTS
RANK
DATA BANKS

*Monitoring points According to program relevance and needs, determine further action and channel through next phases

†Through studies on the penetration of the agents into the organism, their transport, retention, and elimination, their metabolic conversion to the proximate carcinogen the cellular and tissue factors required for such processes, the penetration into target cells and interaction with cell constituents, the neoplastic transformation, and the factors required for the growth of transformed cells into tumors

CHEMICAL CARCINOGENESIS AND PREVENTION OF CANCER (SUMMARY OR PROGRAM PLANS).

E. *FISCAL SUMMARY*

The following charts and tables report the fiscal history and the present distribution of funds in the Carcinogenesis Program, including Direct Operations and Contracts:

Fiscal History
Allocations - Direct Operations and Contracts
Distribution of Contract Funds
Table I - Analysis of Contract Activity by Segment
Table II - Analysis of Contracts by Activity

CARCINOGENESIS PROGRAM - FISCAL HISTORY

DESCRIPTION	JUNE 1968	JUNE 1969	JUNE 1970	JUNE 1971	JUNE 1972	JUNE 1973	JUNE 1974 PROJECTED
PERSONNEL [1]	109	102	106	126	180	167	173
(FULL-TIME PERMANENT)	(107)	(98)	(96)	(110)	(145) [3]	(138)	(140)
(OTHER FULL TIME)	(1)	(1)	(8)	(9)	(23)	(10)	(11)
(PART TIME)	(1)	(3)	(2)	(7)	(12)	(19)	(22)
SPACE (IN SQ. FT.)	15,885	26,829 [2]	26,112	28,337	31,041 [4]	38,164 [5]	39,464
LEVEL OF EXPENDITURE OF IN-HOUSE OPERATION	$1,636,000	$1,802,600	$1,997,000	$2,340,000	$3,538,000	$4,212,000	$4,269,000
NUMBER OF CONTRACTS	33	40	51	69	133	154	173
LEVEL OF EXPENDITURE OF CONTRACTS	$5,602,000	$8,466,600	$7,080,000	$10,598,000	$21,985,813	$26,964,819	$31,800,000 [6]

1 INCLUDES ALL PERSONNEL ACTUALLY ASSIGNED TO THE CARCINOGENESIS PROGRAM.

2 IN NOVEMBER 1968, OFFICES OF THE OADC, LOCATED IN THE FEDERAL BG., AND OFFICES AND LABORATORIES OF THE BIOLOGY BRANCH, LOCATED IN THE AUBURN BG., MOVED TO BG. 37. IN AUGUST 1968, OFFICES AND LABORATORIES OF THE CHEMISTRY BRANCH, LOCATED IN THE AUBURN BG., MOVED TO BG. 37.

3 POSITIONS FOR TWO-YEAR STAFF AND RESEARCH ASSOCIATES FROM MATCHING PROGRAM WERE TRANSFERRED FROM PROGRAM LEVEL TO DIVISION LEVEL.

4 THIS AMOUNT INCLUDES SPACE ACQUIRED FROM THE REGISTRY OF EXPERIMENTAL CANCERS WHICH WAS TRANSFERRED FROM THE OD, NCI, AND THE SPACE ACQUIRED IN THE EYE RESEARCH FOUNDATION.

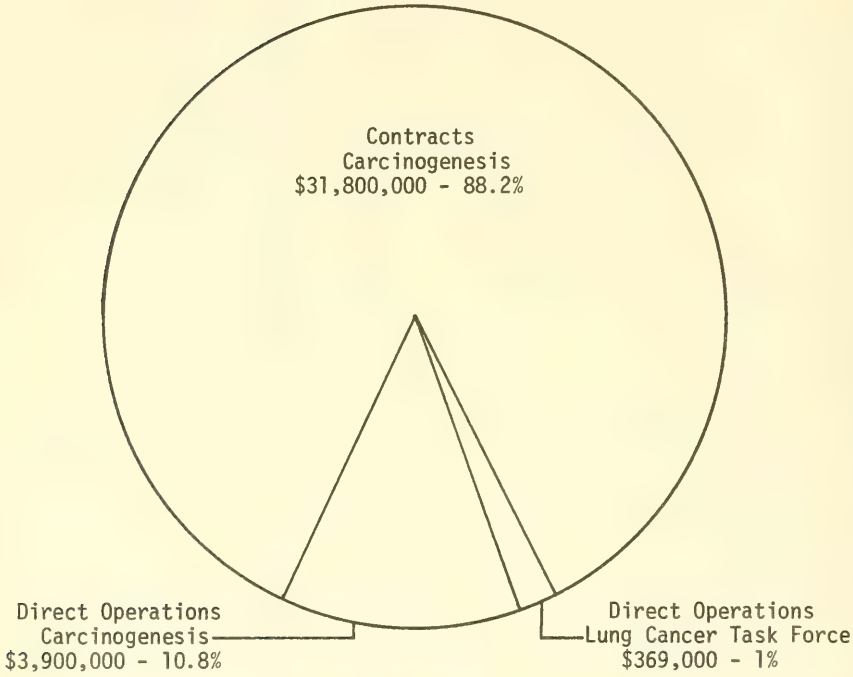
5 IN DECEMBER 1972, OFFICES OF THE OADC, NOT ENGAGED IN LABORATORY WORK, LOCATED IN BG. 37, THE EYE RESEARCH FOUNDATION, AND THE FEDERAL BG., MOVED TO THE LANDOW BG.

6 INCLUDES $4,000,000 FROM IMPOUNDED FUNDS.

CARCINOGENESIS PROGRAM

Allocations - Direct Operations and Contracts[1]

Fiscal Year 1974



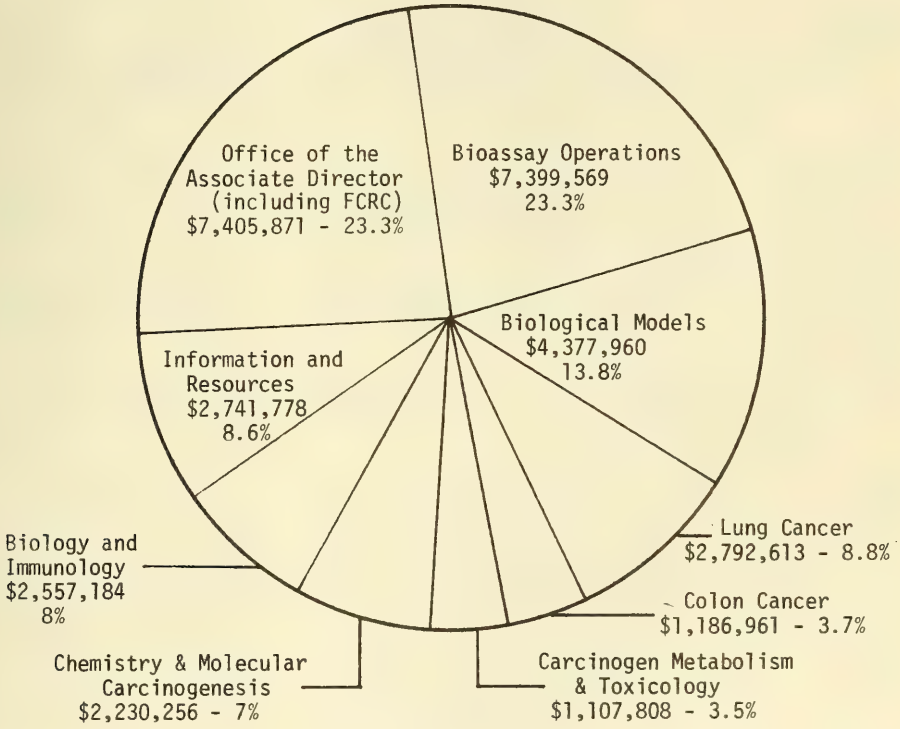
Total Contracts:	$31,800,000
Total Direct Operations:	4,269,000
TOTAL FUNDING:	$36,069,000

[1]Contracts funded from the Lung Cancer Task Force have been transferred to the new Program on Smoking and Health, Office of the Director, DCCP, NCI, for this fiscal year.

CARCINOGENESIS PROGRAM

Distribution of Contract Funds[1]

Fiscal Year 1974


Office of the Associate Director (including FCRC) $7,405,871 - 23.3%
Bioassay Operations $7,399,569 23.3%
Biological Models $4,377,960 13.8%
Information and Resources $2,741,778 8.6%
Biology and Immunology $2,557,184 8%
Chemistry & Molecular Carcinogenesis $2,230,256 - 7%
Carcinogen Metabolism & Toxicology $1,107,808 - 3.5%
Colon Cancer $1,186,961 - 3.7%
Lung Cancer $2,792,613 - 8.8%

Total Contract Funds: $31,800,000

[1]Based on information available as of March 1, 1974

TABLE I

ANALYSIS OF CONTRACT ACTIVITIES BY SEGMENT
IN THE CARCINOGENESIS PROGRAM

SEGMENT	NO. OF CONTRACTS	TOTAL AMOUNT[1]	PERCENT
TOTAL	169[2]	$31,800,000	(100%)
1. OADC	6	7,405,871	23.3
2. Bioassay Operations	22	7,399,569	23.3
3. Biological Models	33	4,377,960	13.8
4. Biology and Immunology	20	2,557,184	8.0
5. Carcinogen Metabolism and Toxicology	10	1,107,808	3.5
6. Chemistry and Molecular Carcinogenesis	25	2,230,256	7.0
7. Colon Cancer	11	1,186,961	3.7
8. Information and Resources	23	2,741,778	8.6
9. Lung Cancer	19	2,792,613	8.8

[1]Estimation based on information available as of March 1, 1974.
[2]Several contracts are listed more than one time.

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT [1]
1. OADC		($ 7,405,871)
AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge Natl. Lab.) Y01-CP-40013	NCI-AEC Carcinogenesis Program [3]	
COLUMBIA UNIVERSITY 72-3234	Development of a Tissue Culture Transformation System for Aromatic Amine Carcinogens	160,000
INTERNATIONAL AGENCY FOR RES. ON CANCER 72-2076	Etiology of Esophageal Cancer in the Caspian Littoral of Iran	-0-
LITTON BIONETICS, INC. (Frederick Cancer Res. Center) 72-3294	Frederick Cancer Research Center Operation	7,170,871
MEMORIAL HOSPITAL 72-3286	A Study of Oncogenesis and Other Late Effects of Cancer Therapy	75,000
NEBRASKA, UNIVERSITY OF (Eppley Inst. for Res. on Cancer) 33278	A Resource for Carcinogenesis Bioassays and Related Research	-0-
SALK INSTITUTE 33405	Carcinogenesis and Control of Cell Cycle	-0-
2. BIOASSAY OPERATIONS SEGMENT		($ 7,399,569)
CHARLES RIVER BREEDING LABS. 72-2004	Production of Animals - Supplement to DCT Contract	88,944
CINCINNATI, UNIVERSITY OF 73-3202	Study of Carcinogenic and Co-Carcinogenic Properties of Industrial Chemicals	94,828

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT [1]
DOW CHEMICAL CO. 72-3254	Carcinogenesis Bioassay of Environmental Chemicals	22,587
GEORGIA, MEDICAL COLLEGE OF 72-3256	Carcinogenesis Bioassay of Environmental Chemicals	-0-
GULF-SOUTH RES. INST. 43267	Carcinogenesis Bioassay of Pesticides and Other Environmental Chemicals	610,544
HAZLETON LABS. 43245	Carcinogenesis Bioassay of Pesticides and Other Chemicals in the Environment	800,299
HOWARD UNIVERSITY 33266	Chemical and Biological Investigation of Potential Carcinogens from Plants	131,000
IIT RES. INST. 71-2338	Carcinogenesis Bioassay of Chlorinated Dibenzodioxins and Related Chemicals	-0-
LITTON BIONETICS, INC. 43349	Carcinogenesis Bioassay of Environmental Chemicals	349,390
LITTON BIONETICS, INC (Frederick Cancer Res. Center) 72-3294	Project 8 - Large-Scale Bioassay	[2]
MASON RES. INST. 43297	Carcinogenesis Bioassay of Environmental Chemicals	643,525
MIDWEST RES. INST. 72-3270	Analytical Chemistry Resource	-0-
NEBRASKA, UNIVERSITY OF (Eppley Inst. for Res. on Cancer) 33278	A Resource for Carcinogenesis Bioassays and Related Research	[2]
NEW YORK UNIVERSITY 43221	Alkylating Agents as Carcinogens	112,136
NEW YORK UNIVERSITY 33241	Transferred to Smoking & Health Program, OD, DCCP, NCI	113,149

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
PAPANICOLAOU CANCER RES. INST. 72-3253	Bioassay of Potential Carcinogenic Compounds in Six Inbred Strains of Rats	70,565
SAN FRANCISCO, UNIVERSITY OF 73-3229	Studies of Carcinogenicity of Metallo-Organic Compounds	130,000
SOUTHERN RES. INST. 73-3214	Carcinogenicity Studies of Chemo-therapeutic Agents and Related Chemicals	310,992
STANFORD RES. INST. 43272	Combined Effects of Chemical Carcinogens & Other Chemicals (Funded by Biological Models Segment)	-0-
TEMPLE UNIVERSITY (Skin & Cancer Hospital) 43271	UV Photocarcinogenesis: Effects of Depletion of Atmospheric Ozone	165,830
TRACOR JITCO, INC. 43350	Bioassay Operations Prime Contract	3,724,222
WOLF RES. AND DEVELOPMENT CORP. 23302	Information Systems Microfilm Services to CBDS	31,558
3. BIOLOGICAL MODELS SEGMENT		($ 4,377,960)
ALTON OCHSNER MEDICAL FDN. 43229	Carcinogenesis by Radiation Plus Estrogen	120,045
BOSTON UNIVERSITY 72-3297	Controlled Methods for the Delivery of Chemical Carcinogens to the Pancreas	103,500
BROOKHAVEN NATL. LAB. 33329	Synergistic Interaction of Hormones and Neutron Radiation on Mammary Gland Carcinogenesis	-0-

TABLE II (CONTINUED

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
CASE WESTERN RESERVE UNIVERSITY 72-3284	Enhanced Induction of Guinea Pig Pancreatic Adenocarcinoma	105,000
CHICAGO, UNIVERSITY OF 73-3287	Definition of Sensitivity of Carcinogenesis Bioassay Systems	132,500
CHICAGO, UNIVERSITY OF 33376	Route of Carcinogen Administration in Pancreatic Adenocarcinoma Induction	125,000
CHILDREN'S HOSPITAL RES. FDN. 33307	Etiology of Medulloblastoma and Other Brain Tumors	98,000
CORBEL LABS. 72-3299	Provide Animal Holding Facilities and Service	106,000
DARTMOUTH COLLEGE 33378	Enhanced Delivery of Synthetic Nitroso Compounds to the Pancreas in Rats	-0-
HAZLETON LABS. 72-3287	Induction of Adenocarcinoma in Dog Prostate	126,500
KANSAS, UNIVERSITY OF 72-3271	Histogenesis of Guinea Pig Pancreatic Adenocarcinoma	-0-
LITTON BIONETICS, INC. 72-2063	Resource for Preparation and Examination of Experimental Biological Material for Histological and Ultrastructural Study	131,626
MARYLAND, UNIVERSITY OF 43279	Studies of the Histogenesis of Renal Carcinoma	74,702
MASON RES. INST. 72-3238	Uptake and Excretion of Carcinogens in and Their Effect on the Prostate	125,000
MICHIGAN, UNIVERSITY OF 73-3303	Isolation and Purification of Epidermal Chalone	103,500
MICHIGAN, UNIVERSITY OF 73-3331	Organ Culture of the Prostate: A Biological Model	-0-

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
MICROBIOLOGICAL ASSOC. 70-2199	Laboratory Service for Support in Carcinogenesis Bioassay and Related Activities	900,000
NEBRASKA, UNIVERSITY OF (at Lincoln)	Chemical Carcinogen-Induced Noduligenesis and Tumorigenesis in Whole Mouse Mammary Gland Organ Culture	-0-
ORGANIZATION FOR HEALTH RES. (TNO) (Radiological Inst.) 33330	Synergistic Interaction of Hormones and Neutron Radiation on Mammary Gland Carcinogenesis	-0-
PAPANICOLAOU CANCER RES. INST. 72-3288	Induction of Prostatic Adenocarcinoma in the Rat	-0-
PORTLAND STATE UNIVERSITY 43256	Isolation and Maintenance of Normal Human Prostatic Epithelial Cell Lines	48,405
ST. LOUIS UNIVERSITY 33370	Synthetic Nitroso Derivatives as a Means of Concentrating Carcinogens in the Pancreas	-0-
SOUTHWEST FDN. FOR RES. AND EDUCATION 33379	Gonadal Hormone Effects on the Prostate	112,500
STANFORD RES. INST. 43272	Combined Effects of Chemical Carcinogens and Other Chemicals (Transferred to Bioassay Operations Segment)	800,477
TEMPLE UNIVERSITY 73-3262	Biochemical and Morphological Components of Hepatic Carcinogenesis	44,063
TENNESSEE, UNIVERSITY OF 33369	Maintenance of Organ Explants from Rodent Pancreas	-0-
WEST VIRGINIA UNIVERSITY 43230	Relationships of Pituitary Hormones and Androgens on Prostate Metabolism	85,166
WRIGHT STATE UNIVERSITY 43231	Cell Culture Development of Human and Guinea Pig Pancreas Cells	55,976

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
TO BE AWARDED	Pancreatic Cancer Research and Management	150,000
TO BE AWARDED	Endpoints for In Vitro Carcinogenesis	170,000
TO BE AWARDED	Identification of Site of Origin of Human Pancreatic Cancer	150,000
TO BE AWARDED	Hormonal Effects During Perinatal Exposure	250,000
TO BE AWARDED	Susceptibility of Fetal Neonate Tissue to Carcinogens	225,000
TO BE AWARDED	Primary Target Cell in Human Pancreatic Adenocarcinoma	35,000
4. BIOLOGY AND IMMUNOLOGY SEGMENT		($ 2,557,184)
AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge Natl. Lab.) Y01-CP-40013	NCI-AEC Carcinogenesis Program	263,400
BECTON, DICKINSON RES. CENTER 71-2168	Carcinogens as Allergens: Detection of Exposure to Carcinogens by Cell-Mediated Immunologic Reactions to the Carcinogens	-0-
BIOLABS, INC. 73-3282	In Vitro Study of the Nature of Interaction between Chemical and Viral Carcinogens	245,000
BRANDEIS UNIVERSITY 72-3243	Production and Detection of Antibodies to Chemical Carcinogens and Other Small Molecules	108,000
CALIFORNIA, UNIVERSITY (at La Jolla) 33403	Significance and Relationship of Fetoglobulins to the Induction of Hepatomas by Chemical Carcinogenesis	160,000

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
CASE WESTERN RESERVE UNIVERSITY 72-3220	Specific Immunological Unresponsiveness to Chemical Carcinogenesis and Its Influence on Tumorigenesis	79,166
CHILDREN'S HOSPITAL MEDICAL CENTER 73-3270	Effects of Carcinogens on In Vitro Synthesis of Complement Components	99,000
DUKE UNIVERSITY MEDICAL CENTER 33313	Response of Peripheral Blood Monocytes from Patients with Neoplastic Disease to Chemotactic Factors	80,000
ILLINOIS, UNIVERSITY OF 72-3205	Transfer of Tumor Immunity by Cell-Free Extracts of Immune Lymphoid Cells	57,500
MALLORY INST. OF PATHOLOGY FDN. 73-3264	Detection of Carcinoembryonic Antigens in Humans	115,000
OHIO STATE UNIVERSITY RES. FDN. 43276	In Vitro Study of the Nature of Interaction between Chemical and Viral Carcinogens	104,783
SCRIPPS CLINIC AND RES. FDN. 43277	Isolation and Chemical Characterization of Soluble Human Tumor (CEA) Specific Antigens	79,972
SORVALL, IVAN, INC. NIAID-73-2513	Fractionation of BCG Cell Walls: A Search for Chemically-Defined Products with Immunologic Anti-Cancer Activity	-0-
TEMPLE UNIVERSITY 33200	Induction of Malignant Melanoma in Guinea Pigs	78,890
TEXAS, UNIVERSITY OF (at San Antonio) 72-3210	Development of In Vitro Methods for the Detection of Cell-Mediated Immunologic Reactivity to Chemical Carcinogens	76,094
TRUDEAU INST., INC. 72-3221	Tumor Inhibition by Mycobacteria: Standardization of Mycobacteria Preparations	201,379

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
UTAH, UNIVERSITY OF 73-3263	Carcinogenesis Bioassay Resource for Determining the Effect of Chronic Immunosuppression on Physical and Chemical Carcinogenesis	260,000
TO BE AWARDED	Carcinogenesis and Immunity	400,000
TO BE AWARDED	Induction of Colonic Tumors in Nonhuman Primates	50,000
TO BE AWARDED	Induction of Colonic Tumors in Guinea Pigs	50,000
TO BE AWARDED	Preparation of Mycobacterial Cell Wall Fractions	49,000
5. CARCINOGEN METABOLISM AND TOXICOLOGY SEGMENT		($ 1,107,808)
AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge Natl. Lab.) Y01-CP-40013	NCI-AEC Carcinogenesis Program	350,000
ARTHUR D. LITTLE, INC. 33392	Use of Lymphoma Cells In Vitro and in Host-Mediated Bioassays as a Potential Prescreen for Chemical Carcinogens	60,000
CALIFORNIA, UNIVERSITY OF (at San Diego) 73-3232	Pulmonary Tumors in Mice for Carcinogenic and Co-Carcinogenic Bioassay	166,855
COLUMBIA UNIVERSITY 33395	In Vitro and Host-Mediated Microbial Mutagenicity as a Prescreen for Chemical Carcinogens	30,000
MASSACHUSETTS INST. OF TECHNOLOGY 73-3238	Interactions between Diet and Chemical Carcinogenesis: A Bioassay System	103,834
MASSACHUSETTS INST. OF 73-3315	Environmental Occurrence of N-Nitroso Compounds	116,500

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
MASSACHUSETTS INST. OF TECHNOLOGY 43265	Toxicity and Carcinogenicity Associated with Fungal Growth on Foodstuffs	208,025
STANFORD RES. INST. 33394	In Vitro DNA Repair as a Potential Prescreen for Chemical Carcinogens	-0-
TEMPLE UNIVERSITY 65-1029	A Search for Carcinogens among Mycotoxins	22,594
TO BE AWARDED	Development and Application of Methods for Detection of N-Nitroso Compounds in the Environment	50,000
6. CHEMISTRY AND MOLECULAR CARCINOGENESIS SEGMENT		**($ 2,230,256)**
AEC-NCI INTERAGENCY AGREEMENT (California, University of [at Berkeley]) Y01-CP-30211	Molecular Processes Involved in the Carcinogenic Action of Polycyclic Aromatic Hydrocarbons	80,000
AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge Natl. Lab.) Y01-CP-40012	NCI-AEC Carcinogenesis Program	135,000
CHICAGO, UNIVERSITY OF 33385	Organic Synthesis of Cold and Tritiated Polycyclic Hydrocarbon Derivatives	70,000
CONNECTICUT, UNIVERSITY OF (at Storrs) 72-3268	Isolation and Characterization of Non-Histone Proteins Binding DNA	-0-
HOME FOR THE JEWISH AGED 73-3274	Study of Serum Haptoglobin Types in Patients with Carcinoma of the Pancreas	-0-
ILLINOIS, UNIVERSITY OF 72-3303	Temperature Sensitive Mutants in In Vitro Carcinogenesis	160,000

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT [1]
INST. OF CANCER RES. 33367	Nature of the Polycyclic Hydrocarbon: Nucleic Acid Compound in Hydrocarbon Carcinogenesis	-0-
JOHNS HOPKINS UNIVERSITY 73-3276	Regulation of the Heme Moiety of P450 in Relationship to Carcinogen Metabolizing Activity	83,000
MANITOBA, UNIVERSITY OF 43250	Isolation, Purification, and Characterization of Human Prolactin	-0-
MICROBIOLOGICAL LABS. 33388	Organic Synthesis of Cold and Tritiated Polycyclic Hydrocarbon Derivatives	-0-
MIDWEST RES. INST. 33387	Organic Synthesis of Cold and Tritiated Polycyclic Hydrocarbon Derivatives	250,000
NEW YORK UNIVERSITY MEDICAL CENTER 33279	The Isolation, Propagation, and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions	130,000
OHIO STATE UNIVERSITY 43248	Research on Preparation of Polymers Containing Carcinogenic Moieties	39,988
OMNI RES., INC. 33386	Organic Synthesis of Cold and Tritiated Polycyclic Hydrocarbon Derivatives	25,000
ONTARIO CANCER INST. 72-2051	The Isolation, Propagation, and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions	134,976
POLYSCIENCES, INC. 72-3245	Optimizing Electrophoretic Separation of Proteins with New Hydrogels	60,000
TEXAS, UNIVERISTY OF (at Austin) 33384	Growth of Soil Bacteria on Polycyclic Hydrocarbons	53,000
TEXAS, UNIVERSITY OF (M.D. Anderson Hospital and Tumor Inst.) 71-2268	Study of Serum Haptoglobin Types in Patients with Carcinoma of the Pancreas	-0-

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
TEXAS, UNIVERSITY OF (M.D. Anderson Hospital and Tumor Inst.) 43270	Non-Histone, DNA Binding, Proteins from Normal Rat Liver and Chemically Induced Rat Hepatomas	83,550
WEIZMANN INST. OF SCIENCE 70-2217	Study of the Role of Enzyme Induction and Chemical Carcinogenesis	136,242
WEIZMANN INST. OF SCIENCE 73-3220	Alterations in Translation of Genetic Messages Induced by Viruses and Carcinogens	240,000
YALE UNIVERSITY 43238	Selection and Propagation of Haploid and Partial Monosomic Cell Lines	99,500
TO BE AWARDED	Studies on Microsomal Enzyme Systems Metabolizing Aryl Hydrocarbons	350,000
TO BE AWARDED	Selection and Propagation of Somatic Cells Having Specific Physiological Mutations	100,000
7. COLON CANCER SEGMENT		($ 1,186,961)
AMERICAN HEALTH FDN. 71-2310	Experimental Large Bowel Carcinogenesis	439,825
CALIFORNIA, UNIVERSITY OF (at Los Angeles) 33380	Fecal Flora Studies	162,000
GEORGIA, MEDICAL COLLEGE OF 43282	Epidemiologic Study of Colon Cancer Among Blacks	63,315
KAISER FDN. RES. INST. 73-3215	Epidemiologic Study of Colon Cancer in Blacks	26,415
LITTON BIONETICS, INC. (Frederick Cancer Res. Center) 72-3294	Project 7 - Laboratory Studies of Bowel Cancer Etiology	[2]

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT [1]
MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES 72-2041	Regulatory Control of Cell Proliferation in Colonic Tissue (in Familial Polyposis)	123,000
MISSOURI, UNIVERSITY OF 33335	Effect of a High Meat Diet on the Bacterial Flora and Chemical Components of Feces	120,000
OMNI RES., INC. 33244	Preparation of Bile Acids and Their Derivatives	-0-
OXFORD, UNIVERSITY OF 72-3215	Study of the Relationship in Individuals between Colon Cancer and Epidemiologically Linked Diseases	-0-
VIRGINIA POLYTECHNIC INST. 12427	Fecal Flora Studies (Supplement to Field Studies and Statistics Program's Contract)	150,000
TO BE AWARDED	Characterization of Mucosal-Associated Microorganisms in the Human Colon and Relationship to Cancer and Precancerous Conditions	75,000
TO BE AWARDED	Epidemiologic Study of Colon Cancer in Blacks - Case Control Identification and Interviewing	27,406
8. INFORMATION AND RESOURCES SEGMENT		($ 2,741,778)
AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge Associated Universities) Y01-CP-40013	Spontaneous Colonic Cancer in the Marmoset	10,000
AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge Natl. Lab.) 72-203	Environmental Mutagen Information Center	80,000
ASH STEVENS, INC. 72-3293	Synthesis of Purine and Pyrimidine Nucleotides	75,000

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT [1]
FRANKLIN INST. RES. LABS. 73-3309	Carcinogenesis Abstracts, Vol. 11	-0-
FRANKLIN INST. RES. LABS. 43268	Information Resources	99,882
IIT RES. INST. 73-3296	Preparation and Characterization of Particulate Materials for Studies in Respiratory Carcinogenesis	57,000
IIT RES. INST. 33383	Stability of Benzopyrene on Particulates Utilized in Respiratory Tract Studies	40,000
INST. OF LAB. ANIMAL RESOURCES (NAS)	Partial Support for the Inst. of Lab. Animal Resources	-0-
INTERNATIONAL AGENCY FOR RES. ON CANCER 43273	Program on the Evaluation of Carcinogenic Risk of Chemicals to Man	15,000
LITTON BIONETICS, INC. (Frederick Cancer Res. Center) 72-3294	Project 9 - Preparation and Characterization of Carcinogens	[2]
LITTON BIONETICS, INC. (Frederick Cancer Res. Center) 72-3294	Project 12 - Production of Inbred and Hybrid Laboratory Animal Strains	[2]
LITTON BIONETICS, INC. (Frederick Cancer Res. Center) 72-3294	Project 15 - Procedures for Safe Handling of Chemical Carcinogens	[2]
MASSACHUSETTS GENERAL HOSPITAL 73-3297	Atlas on Comparative Morphology and Classification of Spontaneous Neoplasma in Dog, Cat, and Man	10,000

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
MEDIZINISCHE HOCHSCHULE HANNOVER 71-2148	Development of a Lung Tumor Model with Large Wild European Hamsters (Cricetus cricetus)	70,000
MOUNT SINAI SCHOOL OF MEDICINE 43255	Identification of Human Populations at Risk Due to Exposure to N-Nitroso Compounds	38,960
STANFORD RES. INST. 33285	Research Program to Acquire and Analyze Information on Chemicals that Have Impact on Man and His Environment	650,000
STARKS ASSOC. 72-3203	Procurement, Purification, Charact-ization and Distribution of Reference Compounds for Carcinogenesis Bioassay (Supplement to DCT Contract)	-0-
TRACOR-JITCO, INC. 33402	Production of 1972-1973 Volume of Survey of Compounds Which Have Been Tested for Carcinogenic Activity and Production of Machine Readable and Searchable Indexes of PHS-149	-0-
WOLF RES. AND DEVELOP-MENT CORP. 43257	Carcinogenesis Bioassay Data System (CBDS) Operational Support (Bioassay Operations Segment)	250,093
TO BE AWARDED	Preparation of Various N-Nitroso Compounds	30,000
TO BE AWARDED	Program Resources	1,000,000
TO BE AWARDED	Animal Pathology Support	90,000
TO BE AWARDED	Animal Pathology Support	105,843
TO BE AWARDED	Carcinogenesis Abstracts, Vol. 12	120,000

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT [1]
9. LUNG CANCER SEGMENT		($ 2,792,613)
AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge Natl. Lab.) Y01-CP-40013	NCI-AEC Carcinogenesis Program	840,000
HARVARD UNIVERSITY SCHOOL OF PUBLIC HEALTH 33273	Factors Influencing Experimental Respiratory Carcinogenesis by Alpha Radiation and Chemical Carcinogens	85,400
IIT RES. INST. 69-2148	Role of Vehicles and Particulates in Respiratory Carcinogenesis Bioassays	-0-
IIT RES. INST. 72-3292	Susceptibility States and Modulating Factors in Respiratory Carcinogenesis	350,000
LITTON BIONETICS, INC. 43274	Studies of Carcinogenesis in Human Bronchial Tissue	106,845
MARYLAND, UNIVERSITY OF 43237	Studies on Normal, Premalignant and Malignant Tracheobronchial Epithelium of Humans	96,880
MASSACHUSETTS INST. OF TECHNOLOGY 69-2083	Role of Vitamin A in the Control of Differentiation and Carcinogenesis in the Respiratory Tract	228,173
MINNESOTA, UNIVERSITY OF 73-3364	Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract	-0-
NEW YORK UNIVERSITY 73-3260	Studies in Pulmonary Carcinogenesis	521,792
OHIO STATE RES. FDN. 69-2144	Study of the Role of Vehicles and Particulates in Respiratory Carcinogenesis Bioassays	-0-
ST. MARY'S HOSPITAL 72-3233	Morphogenesis of Lung Cancer	26,251

TABLE II (CONTINUED)

CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
SOUTHERN RES. INST. 72-2064	Organ Culture and Tissue Culture Bioassay of Vitamin A Analogs	185,000
STANFORD UNIVERSITY SCHOOL OF MEDICINE 43295	Studies on Oat-Cell Carcinoma of the Lung	79,287
STATE UNIVERSITY OF NEW YORK (at Stony Brook) 73-3361	Studies of Carcinogenesis in Organ Culture of Trachea and Bronchi	-0-
STOCKHOLM, UNIVERSITY OF 73-3363	Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract	20,000
TEXAS, UNIVERSITY OF (Southwestern Med. School) 73-3362	Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract	15,000
VERMONT, UNIVERSITY OF 73-3360	Studies of Carcinogenesis in Organ Culture of Trachea and Bronchi	-0-
VETERANS, ADMINISTRATION HOSPITAL (Tampa, Florida) 40204	Autoradiographic Study of the Cellular Response of the Respiratory Tract in Chemical Carcinogenesis	88,570
TO BE AWARDED	Supply of Hamsters Treated with Respiratory Carcinogens for NCI Carcinogenesis Program	149,415
	GRAND TOTAL	$31,800,000

[1]Total funds obligated and estimated through FY 1974.
[2]Total amount reported under Office of the Associate Director for Carcinogenesis.
3Funding is listed under segments sponsoring various tasks at AEC/ORNL

II. SCIENTIFIC ACTIVITIES: PROGRESS HIGHLIGHTS

The progress accomplished in the Carcinogenesis Program in FY 1974 continues to cover a broad front of attack to the problem of identification of causative factors and the prevention of cancers. Many of the projects in this Program have been underway for several years and represent a patient, intensive, and costly long-term effort towards the attainment of our specific program goals. Work in chemical carcinogenesis is characterized to a large extent by the two-three year duration of each lifespan study in common laboratory rodents, with the resulting long-range commitment of efforts and resources. Other projects represent a complex effort to develop and refine the research methods necessary for studying the spectrum of chemical, biochemical, biological, and molecular mechanisms that are responsible for the host response to chemical and physical carcinogens, and for the identification of exploitable preventive measures.

Summary reports of the Office of the Associate Director, of the Branches, and of the Segments, as well as detailed reports of contracts, are presented in the following sections of this Annual Report.

A. *TECHNICAL INFORMATION AND PROGRAM RESOURCES*

Considerable progress has been made in the development of technical information in the area of environmental carcinogenesis and carcinogen bioassay and evaluation. Close liaison has been maintained with other agencies in DHEW, as well as with other departments, agencies and programs in the federal government. This has been accomplished through the DHEW Committee to Coordinate Toxicology and Related Programs, the Interagency Collaborative Group on Environmental Carcinogenesis sponsored by this Program, as well as by direct contacts with industry, labor unions, scientific societies, and individual experts. This effort, in spite of severe limitations in available personnel, has enabled the Carcinogenesis Program to play an increasingly major role in the development of regulatory criteria in the federal government related to exposure to environmental carcinogens.

A major project is devoted to collecting information on production, use, and extent of human exposure for chemicals which have significant contact with man. This project has already provided detailed documentation on 4,000 chemicals (Stanford Research Institute, Information and Resources Segment).

Monographs on the evaluation of individual chemicals for carcinogenesis have been developed by the International Agency for Research on Cancer (IARC) with collaboration from this Program. These monographs have become the major international reference for governments and health agencies. One-hundred and fifty-one compounds have been reviewed so far.

Public Health Service Publication No. 149 entitled "Survey of Compounds Which Have Been Tested for Carcinogenic Activity" has been brought up to date. The 1970-1971 volume has been published and the 1972-1973 volume is in preparation.

In addition, the IARC has initiated the publication of an international registry of compounds undergoing long-term carcinogenesis tests.

The problem of data dissemination in carcinogenesis was recognized as being of the utmost importance particularly for the documentation of bioassays of environmental carcinogens. A workshop was held in January 1974 to discuss methods of presenting carcinogenesis data to the public at large.

The Carcinogenesis Bioassay Data System (CBDS) is now fully operational and receives input for computer storage and analyses from 20 contractors.

Chemical resources have been developed to acquire or when necessary synthesize, characterize, and distribute a variety of materials used for bioassay or as reference substances. This activity has reached such a level that a centralized repository has been established at the NCI's Frederick Cancer Research Center. Guidelines for distribution and priority assignments are being developed.

Safety guidelines for research involving chemical carcinogens have been developed for use by laboratory workers, in coordination with DHEW and the Occupational Safety and Health Administration of the Department of Labor.

Animal resources have been expanded with the acquisition of the large animal farm facility at the NCI's Frederick Cancer Research Center. This has become a single source capable of providing well-characterized, uniform, and healthy animals for a large proportion of our bioassay program.

Guidelines for the long-term holding and care of rodents for carcinogenesis studies have been developed through a workshop held in collaboration with the National Academy of Sciences--National Research Council's Institute of Laboratory Animal Resources.

A special project has provided for the development and characterization of the wild European hamster (*Cricetus cricetus*) which was found to be a valuable experimental animal for carcinogenesis studies (Medizinische Hochschule--Hannover, Information and Resources Segment).

B. *BIOASSAYS FOR CARCINOGENIC ACTIVITY*

A major effort is presently underway to submit a large number of chemicals to carcinogenicity bioassays in well-defined and standardized experimental conditions. Approximately 450 chemicals are being screened for carcinogenicity under contract from the Bioassay Operations Segment. Included are 92 pharmaceuticals, 91 industrial chemicals, 71 pesticides and agricultural chemicals, 44 metallic compounds, and in addition natural materials, food additives, and a variety of other environmental chemicals and intermediates. The animal population used this year for bioassay operations was approximately 110,000 animals. These include about 50,000 rats of the Osborne-Mendel, Fischer/344 and Sprague-Dawley strains; 55,000 mice of the Swiss, C57Bl/6 and B6C3F1 strains; and 5,000 animals of other species mostly hamsters. Most chemicals are tested by a protocol that includes preliminary toxicity studies followed by life-time tests on groups of 50

animals each in two species, both sexes, and at two dose levels (including the maximum tolerated dose).

The establishment of standardized, detailed protocols for all the major bioassay procedures and for data acquisition and analyses will enable the Program to make an important transition. Until now, our level of activity was mostly aimed at the screening of environmental chemicals to identify suspected carcinogens for further study. We are now preparing to enter into a phase where more emphasis will be given to generating detailed bioassay data at the level of sophistication and control necessary to meet regulatory requirements. Emphasis has been placed on the need for documentation of all data entering into and resulting from a carcinogenesis bioassay.

Carcinogenic activity has been identified for several pesticides and pharmaceuticals. Several substituted hydrazines in widespread environmental use have been found to be markedly carcinogenic for a variety of organs (Litton Bionetics, Inc., Gulf South Research Institute, Southern Research Institute, and University of Nebraska [Eppley Institute for Research on Cancer], Bioassay Operations Segment).

Studies on strain susceptibility indicate that no single strain appears to be the most or least tolerant to a series of test compounds (Medical College of Georgia, Bioassay Operations Segment).

C. *EXPERIMENTAL PATHOLOGY MODELS FOR CANCER INDUCTION AND PREVENTION*

1. Tumor Pathology

A major part of the data representing the results of carcinogenesis tests consists of the pathological evaluation of the lesions in test and control animals. Therefore, the Program needs comprehensive and detailed reference standards for the classification of spontaneous and induced tumors in common laboratory animals and their comparisons with the corresponding tumors in man. A major effort has been made to establish a reference center for tumor pathology in carcinogenesis studies. The newly established Tumor Pathology Section which includes the preexisting Registry of Experimental Cancers, located in the Office of the Associate Director for Carcinogenesis, has considerably expanded this activity. Pathology protocols have been developed in consultation with several leading pathologists. The availability of qualified pathologists under contract as well as in NCI and of funds to cover extensive pathology study of animals under test may prove to be the limiting factors that will determine the extent to which bioassay operations can be carried. The Registry of Experimental Cancers has reached a total of 20,000 records. Selected pathology studies of induced tumors has been carried out throughout the Program.

2. Lung Cancer

Methods have been developed to study morphologic and biochemical events in carcinogenesis directly on the respiratory epithelium, using in vivo or in vitro exposure techniques, in animals and in human tissues. Specific

early ultrastructural and biochemical changes induced by carcinogens have been identified. The role of anticarcinogenic substances is investigated. A major pathway of action of retinol in mucus synthesis has been identified as the control of transglycosylation reactions: its role in the control of respiratory cancer induction is actively studied.

Systems for the generation of aerosols, vapors, and industrial dusts have been developed for testing the carcinogenicity of many substances by the inhalation route. Among the important substances which have been found to be carcinogenic or co-carcinogenic for the respiratory tract are calcium chromate dusts, sulfur dioxide, dusts formed from polyurethane foam (workers in the building trades industry may be exposed to this type of dust), and bis-chloromethyl-ether. The above findings have been reported to the National Institute of Occupational Safety and Health, and efforts have been made to curtail human exposure and have formed the basis for the emission of occupational exposure standards by the Occupational Safety and Health Administration (Department of Labor) (*Federal Register*, January 29, 1974). Long-term follow-up studies of exposed workers have been instituted (New York University, Lung Cancer Segment).

An intensive effort is being made to improve the technique of sputum cytology and to use it effectively for earlier diagnosis of morphological stages in the development of lung cancer in man. A large group of uranium miners is being followed with sputum cytology in order to achieve the most accurate definition of the time and stage of development of lung cancer in this population of workers who are at such high risk for lung cancer. An atlas of human sputum cytology is being compiled that will be of help in diagnostic centers throughout the country. A method for respiratory cytology has been developed for laboratory animals and a good correlation has been found between animals and man, in terms of the cytologic stages that occur during the development of bronchogenic squamous cell cancer. These studies are important because they indicate that findings made in the animal model should be of direct relevance to study of the human disease (St. Mary's Hospital and AEC-Oak Ridge National Laboratory, Lung Cancer Segment).

Another follow-up study on a high-risk population was initiated in collaboration with the NCI's Cancer Control Program and with the National Institute of Occupational Safety and Health for asbestos workers formerly under heavy exposure conditions (East Texas Chest Hospital, Lung Cancer Segment).

Exfoliative cytology studies during the experimental development of bronchogenic carcinoma in hamsters suggest that preinvasive carcinoma can be detected cytologically and that accurate diagnosis of early invasive carcinoma is feasible. This finding is of great importance with respect to the problem of early diagnosis of human lung cancer and for monitoring the effectiveness of inhibitory treatments (AEC-Oak Ridge National Laboratory, Lung Cancer Segment).

The process of development of squamous cell cancer of the lung in the hamster animal model is strikingly similar to that found in man and the ultrastructure of the tumors caused by either of the above two carcinogens in the animal is very similar to the ultrastructure of comparable human

tumors. These findings again indicate that the animal model should be of direct relevance to the human disease. A synergistic increase in tumor response was found in hamsters by exposure to benzo[α]pyrene (BP)-ferric oxide followed by multiple dose of nitrosomethylurea (Lung Cancer Branch).

Preliminary data also suggest synergistic effects of BP with Polonium-210 (Harvard School of Public Health, Lung Cancer Segment). The European hamster was found to be susceptible to the induction of bronchogenic carcinoma and may provide an additional, larger animal model (Medizinische Hochschule--Hannover, Information and Resources Segment).

Short-term organ culture methods developed in the Lung Cancer Branch's laboratories were used to study the binding of carcinogens to target cells in the respiratory tract. Higher binding of BP to DNA was found in strains with higher susceptibility to its carcinogenic action and also in vitamin A deficient animals. Conversely, inclusion of high levels of retinol acetate in the organ culture medium inhibits binding of BP to DNA. Long-term organ culture systems have been established during this year. Good survival of the organ cultures of tracheobronchial epithelium was found both for human and for rodent tissues. It has already been found that human bronchial epithelium can activate BP to a form which is tightly bound to epithelial cells predominately in the nucleus (Lung Cancer Branch).

Binding studies, autoradiographic studies, and cell kinetic studies continue in these systems (Veterans Administration Hospital [Tampa, Florida], Lung Cancer Segment).

The inhibition of carcinogenesis in the respiratory tract by vitamin A has been concerned in a study in rats exposed to methylcholanthrene (AEC-Oak Ridge National Laboratory, Lung Cancer Segment). New synthetic analogs of vitamin A are being tested for their ability to inhibit squamous cell differentiation. Some of these compounds have been found to inhibit microsomal mixed function oxidases and may, therefore, relate to the control of carcinogen metabolism (Southern Research Institute, Lung Cancer Segment). The molecular mechanisms of action of vitamin A were further investigated. It was previously found that tracheal epithelium can synthesize a mannolipid from retinol and GDP-mannose. This mannolipid has the structure of a retinol-phosphate-mannose type of compound and has been shown to function as a carrier for mannose during formation of glycoproteins. Retinol-phosphate has now been chemically synthesized and found to be a good substrate for mannosyl-transferase in an in vitro system.

Mouse skin epidermal monolayers cultured in the presence of vitamin A synthesize a high molecular weight glycopeptide which is not found in normal cultures and which contain both fucose and glucosamine. Vitamin A deficiency decreases the incorporation of labeled fucose and glucosamine into a tracheal epithelial glycopeptide from rat tracheal epithelium.

3. Colon Cancer

A program had been established in FY 1972 in close collaboration with the National Task Force on Colon Cancer. Its focus is on the definition of the correlation of colon cancer morbidity in migrant populations and the pathology of these cancers with environmental dietary factors. The role of bacterial flora in the metabolism of intestinal carcinogens and in the susceptibility of population groups is being studied.

Studies in the Epidemiologic Pathology Unit showed the following results. (a) The previously reported association, based on case-control data, between the colonic cancer and high beef consumption, was supported in an analysis of international cancer mortality and food consumption data. Support also emerged from analyses of U.S. food consumption patterns in relation to cancer incidence and mortality.

(b) Epidemiologic and pathologic data on bowel cancer suggested that while rectal cancer is closely related to colon cancer, there is a second set of rectal cancers of different etiology. This is consistent with differences in location of rectal cancers found in populations of low and high incidence.

(c) The results from the international analysis of food consumption in relation to cancer rates indicated the need for exploration of common etiological dietary factors in the development of cancers of the breast and of the colon.

(d) The assumption of opposing etiologies in the development of colon and stomach cancer was found to be highly questionable.

(e) Although occupational hazards in the etiology of bowel cancer have received little attention, an analysis of British and U.S. occupational data showed elevated death rates within certain manufacturing industries, those concerned with metal work, yarn or textiles, and leather goods.

The general consensus about the cause of human bowel cancer is that bacteria in the large bowel produce the actual carcinogen from precursors in the feces. Microorganisms isolated from feces of subjects on a mixed western diet degraded in vitro both neutral and acid steroids to a greater extent than those obtained from vegetarians. Fewer colon tumors appeared in germ-free rats treated with 1,2-dimethylhydrazine than appeared in conventional rats (American Health Foundation, Colon Cancer Segment). Metabolic studies and cell kinetics studies have progressed. Transplantable colon carcinomas have been established (Carcinogen Metabolism and Toxicology Branch).

4. Pancreas Cancer

Considerable progress has been made in the group of projects on the pathogenesis of cancer of the pancreas. Within 36 weeks of treatment with methylnitrosourea, pancreatic adenocarcinoma has been found in 36% of strain 13 guinea pigs. This markedly reduced latent period and increased incidence from previously reported studies is an important step

in the development of a useful animal model for this neoplasm. Nitrosamide carcinogens appear to be bound to RNA and DNA in guinea pig pancreas. Uptake of these carcinogens appears greater in the head than in the tail of this organ (University of Kansas and Case Western Reserve University, Biological Models Segment). Preliminary reports suggest that a high incidence of pancreas tumors can also be obtained in hamsters by a nitrosamine derivative (University of Nebraska [Eppley Institute for Research on Cancer], Office of the Associate Director for Carcinogenesis).

Cell and organ culture methods for pancreas studies have been well established. A study of serum heptaglobin types in patients with carcinoma of the pancreas shows an increased incidence of type 1-1 haptoglobin in these patients when compared with properly selected controls (Home for the Jewish Aged, Chemistry and Molecular Carcinogenesis Segment).

5. Other Organ-Oriented Programs

In mammary carcinogenesis, an interesting, new model for the induction in organ culture of aveolar nodular structures resembling precancerous nodules seen in vivo has been reported (University of Nebraska [at Lincoln], Biological Models Segment). Additional efforts continue in studies of the pathogenesis of cancer of the prostate, kidney, liver, skin and trans-placentally induced cancers, particularly of the nervous system.

6. In Vitro Models for Chemical Carcinogenesis Studies

Considerable progress has been made in this area which is becoming one of the most productive and exciting field of development in carcinogenesis studies. The transformation by chemicals in vitro of hamster embryo cells and of guinea pig embryo cells was previously reported. It has now been demonstrated that rat embryo cells from random-bred, germ-free and SPF rats can be transformed by chemicals in vitro. Several cell strains derived from freshly isolated diploid guinea pig cells exposed in utero or in culture to chemical carcinogens have been obtained. Although morphologic alterations and growth properties of transformed guinea pig cells are similar to those seen with transformation of mouse, rat, and hamster cells, their ability to obtain progressive tumor growth is relatively delayed. Comparative studies of chromosomal changes indicated that neither in vitro transformation nor in vivo tumor induction is accompanied by a specific numerical or structural chromosomal aberration. The synergism of chemical and physical agents or chemical and viral agents or different chemicals in the induction of transformation in vitro has been further documented (Biology Branch).

A number of advances have been made using in vitro cell transformation models in the study of carcinogen metabolism and activation, surface changes, somatic cell genetics, and biochemistry of transformed cells.

Major projects have been initiated to establish well-defined test systems as models for screening environmental chemicals through in vitro cell

transformation tests (AEC-Oak Ridge National Laboratory and NCI's Frederick Cancer Research Center, Office of the Associate Director for Carcinogenesis).

D. *IMMUNOLOGICAL STUDIES IN CARCINOGENESIS*

Radioimmunoassays have been developed for several carcinogens and related compounds (Brandeis University, Biology and Immunology Segment). In a study of carcinogens and immunosuppression, it was found that tumors induced by ultraviolet light exhibit an usually high degree of antigenicity (University of Utah, Biology and Immunology Segment). Considerable progress was made in the study of the components of BCG responsible for tumor cell killing and in the study of the mechanism of action of cell-mediated immunity in this system in several contracts and in in-house research in the Biology Branch.

Refined methods have been developed for measurement of nanogram amounts per ml of serum of carcinoembryonic antigen (Mallory Institute). A sensitive radioimmunoassay was developed also for rat $alpha_1$-fetoprotein and used in the study of liver carcinogenesis (University of California [at La Jolla], Biology and Immunology Segment).

E. *CHEMISTRY, CARCINOGEN METABOLISM, AND MOLECULAR CARCINOGENESIS*

Metabolic studies have been developed for a number of chemical carcinogens both in-house and through contracts.

Metabolism of DDT was studied in several species; DDT was shown to produce lung tumors in mice; both DDD and DDOH were found active in inducing neoplastic transformation of mouse embryo cells. Substantial progress has been made on the metabolism of the environmental carcinogen 2,4-diamino-toluene, a dyestuff and formed plastics ingredient (Carcinogen Metabolism and Toxicology Branch). A major program has been developed in the field of carcinogenesis and metabolism studies of nitrosamines and related compounds. Particular emphasis is given to the processes of nitrosation in the formation of nitrosamines directly in animals or in man and to the metabolic fate of the nitrosamines and their reaction with target cell constituents.

Nitrite has consistently been found in human saliva at levels of 6 ± 6 ppm. Salivary nitrites contribute more to human exposure to nitrites than any other source. Its formation is controlled by the microbial reduction of nitrite from nitrate in the oral cavity.

Other inhibitors of the nitrosation reaction have been found. Kinetics of the nitrosation reaction have been further studied (Carcinogen Metabolism and Toxicology Branch, University of Nebraska, AEC-Oak Ridge National Laboratory, and Massachusetts Institute of Technology).

An extensive effort has been devoted to studies on the polynuclear aromatic hydrocarbons. These involve their mechanisms of interaction and metabolic pathways, the effect of certain co-factors on the metabolism of polynuclear hydrocarbons, and extensive studies on the enzyme systems which control their metabolic detoxification and activation. Among other important findings, it has been observed that bacteria are capable of oxidizing benzo[a]pyrene and benzo[a]anthracene to the dihydrodiol derivatives (University of Texas [at Austin]). More sensitive methods have been developed for the detection of the microsomal enzymes responsible for carcinogen metabolism in human material, such as blood cells. The use of high-pressure, liquid chromatography has been a milestone in the study of metabolism of polynuclear hydrocarbon carcinogens. This technique can separate as many as ten metabolites of benzo[a]pyrene in a short period of time and can therefore correlate carcinogen susceptibility with unique metabolic profiles (Chemistry Branch).

Aryl hydrocarbon hydroxylase activity was found in guinea pig peritoneal and alveolar macrophages. The enzymatic control of carcinogen interaction in macrophages is studied in relation to immunologic sensitization to carcinogens as well as to carcinogenesis target processes (Biology Branch and Chemistry Branch).

The interaction of carcinogens with DNA and DNA-repair mechanisms have been studied in different systems using animal and human cells. Molecular mechanisms of carcinogenesis and of carcinogen interaction have been continued in a variety of systems.

* * *

It would be impossible to summarize all the specific advances made using increasing numbers of research methodologies and biological models at the molecular, cellular, and tissue levels. The reports of the branches and segments as well as the individual project reports presented in the following pages will provide details in all these areas of advances.

III. OFFICE AND BRANCH SUMMARY REPORTS

SUMMARY REPORT

OFFICE OF THE ASSOCIATE DIRECTOR FOR CARCINOGENESIS

July 1, 1973 through June 30, 1974

"(1) Plans, implements, and administers a coordinated program of basic and applied research on carcinogenesis by chemical and physical factors and on its prevention and (2) conducts programs in the areas of (a) coordination on environmental carcinogenesis, (b) epidemiologic pathology, and (c) tumor pathology."

The direct operation of the Office of the Associate Director for Carcinogenesis includes the immediate Office of the Associate Director for Carcinogenesis (U. Saffiotti, Associate Director, and J. A. Cooper II, Deputy Associate Director); the Coordinator for Collaborative Research (A. H. Heim); the Scientific Coordinator for Carcinogenesis at the NCI's Frederick Cancer Research Center (FCRC) (R. R. Bates); the Scientific Coordinator for Environmental Carcinogenesis (H. Kraybill); and the Tumor Pathology Section (R. A. Squire). Until April 1973 this Office also included the Program and Data Analysis Unit (N. Page, Acting Head) and the Lung Cancer Unit (M. B. Sporn, Head). At that time, these two Units were requalified and established as branches--Carcinogen Bioassay and Program Resources Branch (N. Page, Chief) and Lung Cancer Branch (M. B. Sporn, Chief).

The planning and coordination of the whole Carcinogenesis Program has continued to take the major effort of the Office. This Office has continued to serve as a focal point for the development of documentation on the assessment of environmental carcinogenesis hazards and on the principles for evaluation of environmental carcinogens, in collaboration with the Field Studies and Statistics Program, with the Director, DCCP, NCI, and with the Director, NCI. In this capacity this Office has participated in a number of review and policy meetings at the request of other government departments and agencies, including the following: the Office of the Assistant Secretary for Health, DHEW, and particularly its DHEW Committee to Coordinate Toxicology and Related Programs; the Bureaus of Food, of Drugs, and of Veterinary Medicine, FDA; Advisory Groups for the National Center for Toxicological Research, FDA; and several committees and ad hoc groups with FDA, EPA, NIOSH, OSHA (DoL), and other agencies.

Scientific evaluation was provided and testimony was prepared by the Associate Director at the request of the EPA for the hearings on the pesticides, mirex, aldrin and dieldrin.

The Office participated in the activities of the Committee on Environmental Carcinogens and of the Working Group on the Evaluation of Carcinogenic Risk of Chemicals to Man, International Agency for Research on Cancer (WHO), Lyon,

France, and the Scientific Group on the Assessment of the Carcinogenicity and Mutagenicity of Chemicals, WHO, Geneva, Switzerland. This Office also contributed to the organization of several meetings as reported in the Carcinogenesis Program Summary Report.

These and other related activities in the area of evaluation of environmental hazards have required a large share of the resources of this Office, including communication with congressional committees, other government agencies, the press, and the information offices at NCI, NIH, DHEW. Major emphasis has been given in all these activities to the unique role of NCI in providing documentation of carcinogenesis bioassay studies and scientific criteria for evaluation of carcinogenic hazards for man.

The major effort of this Office has been devoted to the scientific planning, development, coordination, and implementation of resources and activities assigned to the Carcinogenesis Program.

Office of the Scientific Coordinator for Environmental Carcinogenesis - The major goal of this organizational unit is to provide mechanisms for coordinating efforts, either intramurally or extramurally, which relate to the impact of environmental systems (agents) on potential induction of human cancer. In this effort it is essential to obtain the collaboration of those other governmental agencies having programmatic responsibilities in environmental monitoring, surveillance, and environmental health and controls. The environmental stresses may encompass those introduced as contaminants or pollutants in the air, water, and diet, and the more intensive exposures received in the work place (occupational exposure). Also, there are data and information resources beyond NCI and other governmental agencies. They are industrial organizations, trade and manufacturing associations, and academic research institutes, which release environmental information as it relates to carcinogenesis.

This coordinative function, while focusing upon the efforts of single etiologic agents (industrial chemicals, agricultural chemicals, food contaminants and additives, natural toxins, drugs, physical or parasitic agents), is concerned more with a total systems approach. Specifically, investigations are concerned with the spectrum of contaminants that occur in man's air, his potable water, his workplace, and his diet, since his daily exposure is really an integration of all of these exposure modes.

The provisions of the National Cancer Act of 1971 and of the National Cancer Plan emphasize the need for a coordinated effort in a national program on environmental carcinogenesis. The program requires data from experimental resources (animal data on chemical exposure and response), monitoring and surveillance (definition of chemical agents, levels of exposure, geographic locations and trends in exposure), and finally the input from human population laboratories (demography, epidemiology and statistics) and leads from epizootics (e.g. diseases and tumors in aquatic animals).

To provide a resource on environmental data relevant to carcinogenesis, there was a need for establishment of a liaison between NCI and other federal and state agencies, industrial groups, and private organizations.

Human cancers are likely to be induced by the ever-increasing exposures which man receives in his environment and his work place. Monitoring and surveillance programs and the sophisticated analytical methodology are available not only to identify the many unwanted contaminants in our environment, but also their levels of occurrence and, in many instances, their many-fold increases. There is a background of naturally-derived pollutants (natural plant toxins, bacterial toxins, fungal toxins), radiation (ultraviolet and ionizing), geologically-eroded minerals which contaminate the air we breathe, the water we drink and are biomagnified in the food chain to the foods consumed. Obviously, efforts must be made to identify, monitor, and control those effluents from agricultural and industrial operations into our environment, which may singly or in combination lead to cancer induction.

Initially, interagency collaboration was designed primarily as a resource for data input, but it became evident that such collaboration would not only comply with recent mandates of the National Cancer Program, but it became a useful mechanism to alert other agencies on cancer problems. In turn, they alerted NCI as to environmental problems which needed NCI assistance for research, expert evaluation, and information. NCI is advisory and not regulatory, but agencies with the latter function can refer to NCI for assistance and advice.

The Interagency Collaborative Group on Environmental Carcinogenesis, established last year, is now operating as a well-established function of this office, carrying out a program responsibility of NCI. There are, at present, 18 agency or subagency groups involved in this collaborative effort. Among those participating from the regulatory area are (1) Consumer Product Safety Commission, (2) Environmental Protection Agency (air, water, pesticides, toxic substances, and radiation), (3) Food and Drug Administration - Bureaus of Drugs and of Foods (food, drug, environmental agents), (4) Department of Labor/Occupational Safety and Health Administration (occupational exposures), (5) Atomic Energy Commission (environmental pollutants - air and water, and radiation controls), (6) Department of Agriculture (environmental/agricultural pollutants), (7) Department of Transportation (environmental/occupational transport of hazardous chemicals). Those with a non-regulatory mission are (8) DHEW/NIH/National Cancer Institute - Cancer Control Program, Division of Cancer Cause and Prevention, (9) DHEW/NIH/National Library of Medicine (Toxicon Program), (10) DHEW/NIH/National Institute of Environmental Health Sciences (environmental), (11) DHEW/CDC/National Institute of Occupational Safety and Health (occupational exposure), (12) Smithsonian Institution (aquatic environment), (13) National Science Foundation (environmental), (14) Department of Interior - Fish and Wildlife Service (aquatic environment), (15) National Aeronautics and Space Administration (environmental/occupational), (16) Department of Commerce/National Oceanic and Atmospheric Administration (atmospheric/oceanic pollutants), (17) Department of Defense/Army - Edgewood Arsenal and Walter Reed Army Institute for Research (environmental and occupational), and (18) Department of Labor/Occupational Safety and Health Administration (occupational).

Since inception (January 24, 1973), the Interagency Collaborative Group on Environmental Carcinogenesis (ICGEC) has met regularly, about every six weeks;

by the end of this fiscal year, some ten meetings will have been held with 30-40 participants and observers at each meeting. This office has therefore become a useful focal point for interagency communication and assistance in problem solving relating to environmental and occupational carcinogens.

Dating back to the initiation of the ICGEC, the environmental problems discussed by the Group include the following: (1) legal responsibilities of various federal agencies in the area of carcinogenesis, (2) systems for development of data on production and exposure (monitoring and surveillance), (3) OSHA standards and guideline for occupational exposure to carcinogens, (4) Toxic Substances Lists of various agencies, (5) Toxicon Program (NLM), (6) information systems of various agencies, (7) Registry of tumors in lower animals, (8) AEC - thermal discharge and environmental pollutants, (9) NCI programs of DCCP and Cancer Control, (10) use of animal excreta products in feed (USDA), (11) Department of Transportation problem on the transport of carcinogens and hazardous chemicals, (12) chemical control of sea lamprey in the Great Lakes, (13) the "dioxin" problem (EPA), (14) occupational carcinogens (NIOSH), (15) NCI - biohazard control in cancer research, (16) Toxic Substances Control Act, (17) NCI Chemical Selection Committee - interagency input for chemicals to be bioassayed, (18) carcinogens in waterways and potable water (NCI/EPA), (19) Mutagenesis Program (NIEHS/NCI), (20) Consumer Product Safety Commission program, (21) short-term bioassays for carcinogens (NCI), (22) compendium on pesticides (EPA), (23) the problem of asbestos in water (FDA/EPA), and (24) the vinyl chloride problem (OSHA, NIOSH, NCI).

A considerable amount of time and effort has been contributed to the Department of Labor/OSHA Advisory Committee activities on the review of the Emergency Temporary Standards for the 14 occupational carcinogens. The office has participated on two subcommittees of the DHEW Committee to Coordinate Toxicology and Related Programs, one dealing with the drafting of departmental standards on the use of chemical carcinogens in the laboratory, and the other on development of protocols for a large study on ingested asbestos in experimental animals. This office is also represented on the NCI Cancer Research Safety Committee. Assistance was given to the Bioassay Operations Segment in diliberations on protocols for bioassay and implementation of prime contractor mechanism. This office has also provided project officer functions on two bioassay contract projects (pesticides and industrial chemicals).

The current restrictions on personnel ceilings have made the work of this office considerably difficult and have imposed a severe limitation to its effectiveness.

Epidemiologic Pathology Unit - The head of this Unit, Dr. John W. Berg, left NCI at the very beginning of this fiscal year. The epidemiologist, Dr. Margaret Howell, retired a few months later. Thus only one professional was left in the Unit (Dr. Silverman, microbiologist). Because of reduced personnel ceilings and the consequent impossibility to replace these members of the staff, it was decided to phase out the Unit. Doctor Silverman was transferred to the Carcinogen Metabolism and Toxicology Branch to set up a Bacterial Metabolism Unit. Activities of the Unit were terminated at the end of the first half of Fiscal Year 1974.

The primary thrust of the work during the phase-out period was on the completion of manuscripts for publication and on the transfer of the Unit's material and data, such as those on Japanese migrant studies, to appropriate NCI personnel in the Field Studies and Statistics Program, DCCP, NCI. A literature retrieval system, designed specifically for the scientific literature of special interest to the Unit, was also transferred to the Field Studies and Statistics Program.

Case-control studies of bowel cancer, being conducted under contract with the Kaiser Foundation Research Institute and with the Medical College of Georgia were analyzed. Conferences with contractors were held at NCI to complete revision of a diet history questionnaire and to plan details of the data collection phase which both contractors have reached. The OMB clearance of the final revision of the questionnaire was obtained expeditiously so that the data collection was not delayed.

In collaboration with other agencies, the epidemiologist also participated in research planning sessions with representatives of the Field Studies and Statistics Program, the NIMH, and the National Academy of Sciences. Sources of data which would permit study of the relationship between psychiatric illness and cancer risk were explored. A contract with the National Academy of Sciences for a study appeared to be feasible and will be further pursued by staff in Field Studies and Statistics.

One result of this collaboration was the hypothesis that the reported low cancer rates among the psychiatrically ill may not be due to a biochemical effect from either the illness or drugs administered in the course of treatment; rather, low rates may reflect (1) effects of institutionalization in reducing environmental hazards (such as decreased smoking due to expense and prohibited smoking areas and decreased risk of lung cancer) and (2) statistical artifacts in the data such as failure to take into account relatively early mortality from competing causes of death. It is anticipated that this hypothesis will be formulated for publication.

Major findings made by the Unit can be reported as follows: (1) The previously reported association, based on case-control data, between the colonic cancer and high beef consumption, was supported in an analysis of international cancer mortality and food consumption data. Support also emerged from analyses of U.S. food consumption patterns in relation to cancer incidence and mortality. (2) The results from the international analysis of food consumption in relation to cancer rates indicated the need for exploration of common etiological dietary factors in the development of cancers of the breast and of the colon. (3) Epidemiologic and pathologic data on bowel cancer suggested that while rectal cancer is closely related to colon cancer, there is a second set of rectal cancers of different etiology. This is consistent with differences in location of rectal cancers found in populations of low and high incidence. (4) Although occupational hazards in the etiology of bowel cancer have received little attention, an analysis of British and U.S. occupational data showed elevated death rates within certain manufacturing industries, those concerned with metal work, yarn or textiles, and leather goods. (5) The assumption of opposing etiologies in the development of colon and stomach cancer was found to be highly questionable.

Office of the Scientific Coordinator for Carcinogenesis at the NCI's Frederick Cancer Research Center (FCRC) - This office started its function at the beginning of Fiscal Year 1974 with the appointment of Dr. R. R. Bates, formerly Chief of the Experimental Pathology Branch and Director of the Biological Models Segment. This office provides scientific coordination for carcinogenesis programs at FCRC which include the following: (a) coordinating the activity of existing tasks in carcinogenesis at FCRC which include laboratory studies of bowel cancer etiology, large-scale bioassay, preparation and characterization of carcinogens, *in vitro* bioassay methodology for potential carcinogens, production of inbred and hybrid laboratory animal strains, and procedures for safe handling of chemical carcinogens; (b) collaborating with the Scientific Coordinator for NCI at FCRC, the Associate Director for Carcinogenesis, and Carcinogenesis staff in establishing priorities and levels of funding of tasks at FCRC; (c) assuring the establishment and maintenance of standards of excellence in research in the FCRC laboratories through establishment of appropriate review, consultation, and collaborative arrangements and assuring cooperation among members of various tasks in accomplishing their goals; and (d) developing plans for new tasks and particularly a laboratory research program in endocrine carcinogenesis and other areas of carcinogenesis studies.

In the second year of the FCRC, the principal focus of carcinogenesis activities continued to be on the identification of carcinogens either in the environment or developing within the body as a result of metabolism by intestinal tract bacteria.

Project 7 (Laboratory Studies of Bowel Cancer Etiology) has been devoted principally to the study of metabolism of tryptophan and of bile steroids by anaerobic bacteria from the colon. Several metabolites of tryptophan have been found, but kynurenine has not been found among them. Metabolism of steroids is complex and, for example, at least 35 metabolic products of cholic acid have been found from one bacterial species.

Project 8 (Large Scale Bioassay) is testing a variety of environmental chemicals by chronic oral administration in rodents. By the end of the year, it is expected that 30 chemicals will be on chronic test. It has been noted that accurate determination of maximum tolerated dose is not always achieved in the standard six to eight week subacute phase formerly used in NCI protocols. Therefore, this phase of testing has been increased to 90 days.

Project 9 (Preparation and Characterization of Carcinogens) provides support to the other projects for analyses of chemicals to be tested for their purity and stability. Other functions of this project include synthesis of potential carcinogens or their metabolites for use in studies either at FCRC or at other laboratories and the establishment of a chemical repository for the storage and shipping of reference carcinogens and their analogs.

Project 10 (*In Vitro* Bioassay Methodology for Potential Carcinogens) has the goal to develop standardized *in vitro* bioassay methods for detecting carcinogens. Studies with hamster embryo cells have defined some of the

conditions necessary for optimum sensitivity. Additional experiments are underway with human cell lines.

Project 12 (Production of Inbred and Hybrid Laboratory Animal Strains) provides most of the animals used in carcinogenesis research at FCRC as well as a high proportion of those used in other carcinogenesis contracts throughout the country.

Two new projects have been started this year. Project 15 (Procedures for Safe Handling of Chemical Carcinogens) has been established to develop procedures for safe handling of chemical carcinogens in the laboratory. This project will analyze problems and approaches presently used by a variety of laboratories handling chemical carcinogens and then develop new, improved procedures of use not only at FCRC, but at other laboratories throughout the world.

Project 16 (Chemical Carcinogenesis) has been developed to undertake a broad approach to the study of chemical and physical carcinogenesis and basic research pertaining to them. An immunologic project underway throughout the year has been isolating components of BCG and other lipids which enhance the immunologic rejection of tumors. A new program was established toward the end of the contract year to study carcinogenesis in endocrine target organs. Particular emphasis in this project will be placed on examination of factors affecting the susceptibility or resistance of the mammary gland to carcinogenesis and in developing an experimental model for studying prostate carcinogenesis.

Tumor Pathology Section - *"Establishes and maintains a national tumor pathology reference center with emphasis on experimental tumors and their relationship to the human counterpart. The Section provides diagnostic pathology criteria, standardization, and monitoring for the Carcinogenesis Program and develops liaison with the Experimental Pathology Branch and related criteria for the development of experimental tumor models. The Section maintains a registry of experimental cancers and also provides guidance and training in tumor pathology for the program and consultation services to other national and international agencies on animal tumor pathology.*

This Section was established on February 1, 1974, as a new Section in the Office of the Associate Director for Carcinogenesis, although its activity under its new Head, Dr. R. A. Squire, effectively started in August 1973. The existing Registry of Experimental Cancers became a unit within the Tumor Pathology Section and is actively pursuing and expanding its original objectives.

The goals of the Section are to provide the urgently needed pathology support, monitoring and coordination to both the intramural and collaborative programs of the Carcinogenesis area. These include specifically the following: (1) provide diagnostic pathology criteria, standardization and monitoring through the use of uniform definitions, nomenclature and laboratory methods; (2) develop and evaluate morphologic, epidemiologic and pathogenetic criteria for animal tumor models which are relevant to human disease; and

(3) provide guidance and training in tumor pathology for the Program and consultation services to other national and international agencies.

Another major goal is to establish a national tumor pathology reference center for carcinogenesis studies through the activities of the Registry of Experimental Cancers.

The objectives of the Registry are (a) the storage and retrieval of pathology material and data on cancers and other lesions of animals and (b) the use of such information for research and educational purposes. The Registry possesses a large collection of protocols, pathologic material, including histologic slides, paraffin blocks, and gross specimens of spontaneous and induced cancers and other lesions and has a total of 244 single or group accessions from investigators outside of NCI (118 acquired since last year). Approximately 20,000 records have been prepared for coding and have been coded (8000 since last year). The printouts are used by members of the Registry and by investigators who consult the Registry.

Fifty-four investigators have come to the Registry for consultation on single or multiple visits. Three foreign investigators have spent varying periods of time in the Registry studying some of the available material. Efforts are underway to increase the use of the Registry by scientists in the Carcinogenesis's Collaborative Program.

Serial No. NCI-4696
1. Office of the Associate Director for Carcinogenesis, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Evaluation of Carcinogenic Hazards

Previous Serial Number: Same

Principal Investigator: Umberto Saffiotti, M.D.

Other Investigators: Collaboration from staff of the Carcinogenesis Program, DCCP, NCI

Cooperating Units: Collaboration from the staff of the Field Studies and Statistics Program, DCCP, NCI

Man Years:

Total	:	0.6
Professional	:	0.3
Other	:	0.3

Project Description

Objectives:

To examine current knowledge in the field of carcinogenesis and related fields and to identify criteria for the evaluation of carcinogenic hazards by chemical and physical agents and for the prevention of their carcinogenic effects in man. To examine laboratory findings on the biological action of chemicals and to review data on exposures for man in order to formulate a scientific evaluation of their carcinogenic risk.

Methods Employed:

Scientific coordination of intramural and collaborative programs in the Carcinogenesis Program, DCCP, NCI. Convening expert advisory groups. Consultation with other government and international agencies and with members of the scientific community. Review of scientific information. Individual analysis of data collected in this field and particularly results obtained in carcinogenesis experiments.

Major Findings:

A study was made of the carcinogenesis data and of the pathology submitted by the Environmental Protection Agency on the pesticides, aldrin, dieldrin,

and mirex. Several other documentations were studied and reviewed for other Federal agencies (Food and Drug Administration, National Institute of Occupational Safety and Health, and the Department of Labor's Occupational Safety and Health Administration).

Numerous contributions have been made towards the establishment of national policies in the field of carcinogenesis hazards, through discussions and documentation provided to Congressional Committees, government and international agencies and offices, and to scientific societies.

Significance to Biomedical Research and the Program of the Institute:

The national policies on environmental health and cancer prevention should be based on sound scientific grounds. The Carcinogenesis Program at NCI provides a central source of information and competence in this field.

Proposed Course of Project:

Continuation and expansion of these functions.

Honors and Awards

Member from NCI to DHEW Committee to Coordinate Toxicology and Related Programs

Participant and Representative from the National Cancer Institute at the Policy Board Meetings and observer to the Science Advisory Board meetings of the National Center for Toxicological Research, Food and Drug Administration

Member, Intra-agency Committee to the Board of Advisors of the International Advisory Committee to the Fogarty International Center

Councillor, Society for Occupational and Environmental Health and Member of the Committee on Publications and Awards

Member, Ad Hoc Committee on Smoking and Health, National Cancer Advisory Board, National Cancer Institute

Member, Ad Hoc Advisory Committee for the Frederick Cancer Research Center, National Cancer Institute

Chairman, Carcinogenesis Contract Program Management Group, Division of Cancer Cause and Prevention, National Cancer Institute

Chairman, Carcinogenesis Intramural Program Group, Division of Cancer Cause and Prevention, National Cancer Institute

Member, Cause and Prevention Executive Staff, Division of Cancer Cause and Prevention, National Cancer Institute

Member, Editorial Board, Department of Health, Education, and Welfare Publication No. 149 entitled "Survey of Compounds Which Have Been Tested for Carcinogenic Activity"

Member, Tobacco Working Group, National Cancer Institute

Advisor, Scientific Group on the Assessment of the Carcinogenicity and Mutagenicity of Chemicals, World Health Organization, Geneva, Switzerland, August 13-18, 1973

Chairman, Session on the Relation between Mutagenesis and Carcinogenesis, First International Conference on Environmental Mutagens, sponsored by the Environmental Mutagen Society, Asilomar, California, August 29-September 1, 1973

Participant, Advisory Committee on Environmental Carcinogens, International Agency for Research on Cancer, World Health Organization, Lyon, France, October 15-16, 1973

Chairman, Working Group on the Evaluation of Carcinogenic Risk of Chemicals to Man, International Agency for Research on Cancer, World Health Organization, Lyon, France, October 22-29, 1973

Guest Lecturer, Club de Carcérogénèse, Laboratorie Curie, Paris, France, October 20, 1973

Participant, Conference on Mechanisms of Chemical Carcinogenesis sponsored by the Joint U.S.-Japan Panels on Methods for Evaluating Environmental Mutagenesis and Carcinogenesis of the U.S.-Japan Cooperative Medical Science Program, Charleston, South Carolina, November 19-21, 1973

Invited Lecturer, Local Section of the Society for Experimental Biology and Medicine, Bethesda, Maryland, January 17, 1974

Invited Speaker, Symposium on Environmental Determinants of Human Cancer, and Chairman, Carcinogenesis IV Session, Annual Meeting of the American Association for Cancer Research, Houston, Texas, March 28-30, 1974

Invited Speaker on "Carcinogenesis of Laryngeal Carcinoma", Section on Basic Oncological Research, Centennial Conference on Laryngeal Cancer, Toronto, Canada, May 26-31, 1974

Lecturer, Postgraduate Course on Clinical Oncology (Lung Cancer), Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy, June 3-7, 1974

Participant, Conference on Experimental Respiratory Carcinogenesis and Bioassays, sponsored by Battelle Pacific Northwest Laboratories, Battelle Seattle Research Center, Seattle, Washington, June 24-27, 1974

Serial No. NCI-4696

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Saffiotti, U.: Metabolic host factors in carcinogenesis. In *IARC Monogr.* (Proceedings of the Conference on Host-Environmental Interactions in the Etiology of Cancer in Man--Implementation in Research 1972). (In Press).

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Serial No. NCI-4543
1. Office of the Associate Director for Carcinogenesis, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Epidemiologic and Pathological Studies on Bowel Cancer

Previous Serial Number: Same

Principal Investigators: John W. Berg, M.D. and Margaret A. Howell, Ph.D.

Other Investigators: None

Cooperating Units: J. David Godwin (Biometry Branch, FSS, DCCP, NCI), Grant Stemmermann (Kuakini Hospital, Honolulu, Hawaii), Nobuki Sasano (Tohoku University, Sendai, Japan), Edward Mason (University of Iowa, Iowa City, Iowa), Ronald Welch (Louisiana State University, New Orleans, La.), Roland Phillips (Loma Linda Hospital, Loma Linda, Calif.), John Baldwin (Oxford University, London), John Lee (University of Washington, Seattle, Washington), and Manning Feinlieb (NHLI, NIH)

Man Years:

Total	:	.350
Professional	:	.150
Other	:	.200

Project Description

Objectives:

To assemble and analyze bowel cancer data relevant to the NCI's Bowel Cancer Programs.

Methods Employed:

Record abstracting and analysis, microscopic examination, literature, and data file reviews.

Major Findings:

(1) International mortality and incidence data reinforce the recent case-control observation that bowel cancer and beef consumption are closely associated. The correlation between colonic and rectal cancer, reflected in

incidence data is higher than previously reported. A mathematical description of the association can be interpreted as saying that while much rectal cancer is caused by the same factors that cause colon cancer, there is a second set of rectal cancers of different etiology. This is consistent with differences in location of rectal cancers found in populations of low and high incidence. In the U.S. rectal cancer now tends to be upper rectal cancer, with only about twelve per cent of all bowel cancer palpable on digital examination.

(2) Among Seventh Day Adventists, Dr. Phillips recently confirmed previously reported low cancer rates in that population.

(3) An increasing excess of bowel cancer in men as compared with women raised questions about possible occupational hazards. From available American data and more recent British data, a few high risk occupations were identified. Of United States' industries with elevated death rates, those composed of white-collar workers were predominant, but the manufacturing industries as a group also appeared to have an elevated risk. Within manufacturing, clusters of occupations concerned with metal work, yarn or textiles, and leather goods showed elevated death rates for which there were also some parallels in the English data. Other less consistent results raised the possibility of hazards in specific occupations which may be related to dyes, meat handling, smoke inhalation, and chemicals.

Significance to Biomedical Research and the Program of the Institute:

This project was designed to integrate many types of information either to solve small scale problems within the Bowel Cancer Program or to furnish the basis for establishing larger efforts for outside groups.

Proposed Course of Project:

The principal investigators left the NIH; Dr. Berg resigned in July 1973 and Dr. Howell retired in January 1974. The proposed course is to complete the preparation of manuscripts resulting from this project.

Publications

Berg, J. W., Howell, M. A., and Silverman, S. J.: Dietary hypotheses and diet-related research in the etiology of colon cancer. Health Serv. Rep. 88: 915-924, 1973.

Berg, J. W. and Howell, M. A.: The geographic pathology of bowel cancer. Cancer (In Press).

Serial No. NCI-4544
1. Office of the Associate Director for Carcinogenesis, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Food Consumption Patterns in Population Groups Contrasting in Incidence and Mortality for Cancer of the Large Bowel

Previous Serial Number: Same

Principal Investigator: Margaret A. Howell, Ph.D.

Other Investigators: E. Cuyler Hammond, M.D. (American Cancer Society)

Cooperating Units: Department of Agriculture

Man Years:

Total	:	.025
Professional	:	.025
Other	:	.000

Project Description

Objectives:

To determine if population groups differing in incidence and mortality for cancer of the large bowel show dietary differences consistent with findings from a case-control study of Japanese migrants to Hawaii.

Methods Employed:

Food consumption patterns were studied in three sources of dietary information: the questionnaire used by the American Cancer Society (ACS) in its 1959 prospective study of over one million people, published reports of the Department of Agriculture based on dietary surveys, and per capita food consumption across countries, which is available from the Food and Agriculture Organization (FAO) of the United Nations.

Results from the Japanese study suggested meats and particularly beef were dietary items which differentiated sharply between colorectal cancer cases and controls. For this reason primary interest in the present analysis was in the consumption of meat or related dietary components.

From the American Cancer Society's questionnaire, regional differences in food consumption were determined. Figures on meat consumption were also compiled from the Department of Agriculture's publications.

Based on the FAO data, correlations between dietary components and incidence and mortality rates were calculated.

Major Findings:

While findings from the varied sources of information could only be considered suggestive, they tended to reinforce each other and support the findings from the best source, the case-control study. All pointed to meat as a food item associated with the development of colon cancer and suggested that fish was not associated with this type of cancer.

Significance to Biomedical Research and the Program of the Institute:

Diet is the most suspect of environmental factors affecting the development of cancer of the large bowel. Yet, only one case-control study, that in Japan, has yielded positive findings in the comparison on dietary histories of individuals with the disease and those free of it. If similar dietary differences can be confirmed in population groups contrasting in incidence and mortality rates for colorectal cancer, these provide supportive evidence for implicating specific dietary components.

Proposed Course of Project:

In view of the principal investigator's retirement in January 1974, this project is completed.

Publications

Howell, M. A.: Diet as an etiological factor in the development of cancers of the colon and rectum. J. Chronic Dis. (In Press).

Serial No. NCI-4545
1. Office of the Associate Director for Carcinogenesis, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Development of a Diet History Questionnaire for Use in Case-Control Studies of Colon Cancer

Previous Serial Number: Same

Principal Investigator: Margaret A. Howell, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total	:	.200
Professional	:	.100
Other	:	.100

Project Description

Objectives:

To develop a diet history questionnaire of suitable length for case-control interviews with content appropriate to the study of cancer of the large bowel.

Methods Employed:

Techniques for eliciting diet history information were reviewed, and the most suitable methods for recall were selected for use in a questionnaire. A pool of dietary items was initially developed through (1) a review of the epidemiologic literature to assure adequate item coverage of major dietary hypotheses concerning colorectal cancer, (2) identification and inclusion of items differentiating between colorectal cases and matched hospital controls in a questionnaire used by the Biometry Branch, Field Studies and Statistics Program, DCCP, NCI, in studies in Hawaii, (3) inclusion of items from other research which were suggestive of differences either in case-control studies or population-based studies, and (4) review of items in other diet questionnaires, particularly those used by Department of Agriculture in order to increase the comprehensiveness of coverage of foods.

An initial pool of demographic items was also screened from the epidemiologic literature pertinent to cancer of the large bowel. Refinement, rephrasing, and final selection of items to determine the content of the questionnaire were undertaken as a collaborative effort between the principal investigators and their co-workers involved in case-control contract work.

Preliminary "try-outs" on small number of interviews in two contract locations provided the basis for several revisions of the questionnaire. A final revision resulted from pretesting of more than 50 interviewees.

Major Findings:

Pretest results suggested the questionnaire was of appropriate length, easily understood by interviewees, and likely to yield reliable data.

Significance to Biomedical Research and the Program of the Institute:

Since food is considered the most likely source of environmental carcinogens affecting development of cancers of the colon and rectum, a standard, well-developed diet history questionnaire is important. The collaborative effort has meant greater comparability in information from different locales. The questionnaire can be made available, upon request and with appropriate clearances, for use by other investigators.

Proposed Course of Project:

In view of the principal investigators retirement in January 1974, this project will be continued in the Colon Cancer Segment of the Carcinogenesis Collaborative Program. Through contracts at the Medical College of Georgia (72-3280) and the Kaiser Foundation Research Institute (73-3215), the questionnaire is currently in use for the collection of case-control data.

Serial No. NCI-4546
1. Office of the Associate Director
for Carcinogenesis, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Factor Analysis of Mortality and Incidence Data

Previous Serial Number: Same

Principal Investigator: Margaret A. Howell, Ph.D.

Other Investigators: John W. Berg, M.D.

Cooperating Units: None

Man Years:

Total	.400
Professional	.200
Other	.200

Project Description

Objectives:

Through the use of factor analysis, to search for epidemiologic clues to the etiology of cancer of the various organ sites, with particular emphasis on cancer of the large bowel.

Methods Employed:

Mortality data from 41 countries, states within the United States, and prefectures in Japan were analyzed in an exploratory effort to determine if the factors identified would suggest new etiological hypotheses which could be tested by other methods. Similar analyses were made of available incidence data. For the international analyses, food consumption figures were also considered.

Major Findings:

The analyses revealed factors which were generally interpretable in terms of what is known concerning cancer epidemiology rather than yielding new hypotheses. The results, however, cast doubt on the assumption of opposing etiologies for stomach and colonic cancers, but supported an association between colonic cancer and a high meat diet. The very similar correlational

pattern of breast and colonic cancers with food consumption figures suggested the possibility of common dietary factors in the etiology of cancers of these sites.

Significance to Biomedical Research and the Program of the Institute:

Confirmation or negation of associations which have been presumed to have etiologic significance is an important contribution to orderly progress in the accumulation of scientific information. A review, currently underway, of various kinds of epidemiologic evidence for common etiologic factors in breast and colonic cancers may stimulate new research in this area.

Proposed Course of Project:

The principal investigator retired in January 1974. The proposed course is to complete the preparation of manuscripts resulting from this project.

Publications

Howell, M. A.: Colonic and stomach cancers: Opposing aetiologies? Lancet 2: 1338, 1973.

Howell, M. A.: Factor analysis of international cancer mortality data and per capita food consumption. Br. J. Cancer (In Press).

Serial No. NCI-4548
1. Office of the Associate Director for Carcinogenesis, DCCP
2. Tumor Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Registry of Experimental Cancers

Previous Serial Number: Same

Principal Investigators: Dr. Harold L. Stewart, Consultant
Dr. Thelma B. Dunn, Consultant
Dr. Margaret D. Barrett (Dr. Margaret K. Deringer)

Other Investigators: None

Cooperating Units: None

Man Years:

Total	:	4.0
Professional	:	2.0
Other	:	2.0

Project Description

Objectives:

(1) The storage and retrieval of pathological material and data on cancers and other lesions of laboratory animals (primarily rodents), and (2) the use of such for research and educational purposes.

Methods Employed:

The methods employed in the work of the Registry involve the selection of protocols, pathologic material, including histologic slides, paraffin blocks, and gross specimens; and illustrations in the form of lantern slides, gross photographs and photomicrographs in black and white and in color. The work of the Registry also includes the collection of records of experiments, reprints and references on this material.

The Registry of Experimental Cancers possesses a large collection of spontaneous and induced cancers and other lesions. The pertinent information on the collection is being indexed. Many of the data have been prepared for

and entered into the computer. Printouts have been supplied, upon request, to the Institute of Experimental and Clinical Oncology, Moscow and the European Late Effects Project Group, Munich, W. Germany.

The Registry accesses material from investigators at NCI, other institutes of NIH, other governmental agencies, industrial laboratories, and universities here and abroad. A total of 244 single or group accessions from investigators outside of NCI have been processed since March 1971.

The Registry is preparing Study sets of slides, with explanatory material, relating to particular areas of the pathology of cancer in rodents. A set of small intestine neoplasms has been completed. These sets will be loaned to interested investigators in this country and abroad.

The Registry has 24 Study Sets of slides on "Hematopoietic and Lymphoreticular Neoplasms" (prepared for a workshop on the comparative pathology of these neoplasms at NCI in May 1971) which are loaned, with descriptive material, to investigators who request them. Thirty loans for periods up to two months have been made.

Three investigators have spent varying periods of time at the Registry studying some of the available material. Investigators come to the Registry for consultation. There have been single or multiple consultations with fifty-four individuals since February 1973.

Major Findings:

The functions (outlined in Objectives) of the Registry in the wider field of cancer research and more particularly in the Carcinogenesis program are such that there are no major findings to report.

Significance to Biomedical Research and the Program of the Institute:

The availability of the wealth of material possessed by the Registry advances the knowledge of spontaneous and induced disease processes in animals.

The existence of the Registry will contribute to the standardization of nomenclature of cancers and other lesions in animals.

Proposed Course of Project:

The Registry will continue and expand all of its activities (already set forth in this report.)

The establishment of a formal relationship with the Registry of Experimental Cancers, the European Late Effects Project Group, Mol/Donk, Belgium, and the International Agency for Research on Cancer, Lyon, France, has been proposed.

Serial No. NCI-4548

Publications

Turusov, V. S., Deringer, M. K., Dunn, T. B., Stewart, H. L.: Malignant mouse-liver tumors resembling human hepatoblastomas. J. Natl. Cancer Inst. 51: 1689-1695, 1973.

Stewart, H. L., Dunn, T. B., Snell, K. C., Deringer, M. K.: Tumors and proliferative lesions of the respiratory apparatus in mice. In Turusov, V. S. (Ed.): Pathology of Tumours in Laboratory Animals, Vol. II: Tumours of the Mouse, Lyon, International Agency for Research on Cancer (in press).

Stewart, H. L.: Tumors of the soft tissues In Turusov, V. S.(Ed.): Pathology of Tumours in Laboratory Animals, Vol. II Tumours of the Mouse, Lyon, International Agency for Research on Cancer (in press).

Stewart, H. L. and Snell, K. C.: Patterns of neoplastic and nonneoplastic diseases of Praomys (Mastomys) natalensis In Recent Results in Cancer Research (in press).

Stewart, H. L.: Comparison of sites of multiple neoplastic and atrophic lesions induced by carcinogenic hydrocarbons and fluorenylamine compounds In Severi, L. (Ed.): Multiple Primary Malignant Tumours, Perugia, Division of Cancer Research (in press).

Deringer, M. K.: Multiple primary tumours in breeding females of four strains and three substrains of inbred mice. In Severi, L. (Ed.): Multiple Primary Malignant Tumours, Perugia, Division of Cancer Research (in press).

Serial No. NCI-4397
1. Office of the Associate Director for Carcinogenesis, DCCP
2. Tumor Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Epidemiologic Pathology of Malignant Neoplasms of Man and Animals

Previous Serial Number: Same

Principal Investigator: Carolyn H. Lingeman, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total	:	0.7
Professional	:	0.5
Other	:	0.0

Project Description

Objectives:

To evaluate systematically the epidemiologic, clinical and morphologic characteristics of the most prevalent malignant neoplasms of man and their counterparts in animals in order to better understand the pathogenic and etiologic mechanisms involved in the processes of carcinogenesis.

Methods Employed:

For each of the site-specific cancers selected for study, all relevant information concerning clinical and epidemiologic characteristics of both human and animal neoplasms is being collected from published and unpublished sources. Histologic material from humans and animals with spontaneous and experimental neoplasms of selected sites are being examined to determine if morphologic features are comparable.

Because of the need for efficient means for storing, analyzing and retrieving the large volume of bibliographic information and other data that is accumulated during the course of the study, methods for using data processing systems (WYLBUR and CPS) are being developed.

Major Findings:

There have been no new findings since reports of previous years.

Significance to Biomedical Research and the Program of the Institute:

As part of the commitment of the Carcinogenesis Program to correlate epidemiologic information and laboratory experimentation in evaluating action of environmental carcinogens, this project seeks to provide a like between epidemiologist and laboratory investigator by using the special skills and techniques of the pathologist and morphology as the basis for comparisons.

Proposed Course of Project:

The systematic comparisons of human and animal neoplasms will be continued with increasing attention to searches for valid experimental models.

Publications

Lingeman, C. H.: Liver-cell neoplasms and oral contraceptives. Lancet 1: 64, 1974.

SUMMARY REPORT

BIOLOGY BRANCH

July 1, 1973 through June 30, 1974

"Plans, develops, and conducts a research program aimed at (1) elucidation of the role of cellular and humoral immune responses in the process of chemical carcinogenesis, its inhibition, and the prevention of cancer growth and spread; (2) biochemical characterization and elucidation of the structure of antigens derived from normal and malignant cells; (3) the acquisition of detailed knowledge of the molecular and cellular events required for cell damage by antibodies and complement, by lymphocytes and macrophages, and by a combination of these humoral and cellular factors; and (4) the development and definition of in vitro cellular transformation systems for the rapid detection of the carcinogenic action of chemicals."

Among the stages of carcinogenesis particular attention is paid to 1) transformation of normal to neoplastic cells, 2) multiplication of neoplastic cells to produce a tumor mass, and 3) the formation of metastases. Prevention of any of these stages of cancer development in man would significantly reduce cancer mortality.

The pursuit of this goal in the Biology Branch is based on three areas of research: 1) development of short-term methods of in vitro cell transformation by chemicals to be used to identify and select carcinogenic chemicals, 2) development of immunologic methods to identify and detect exposure of individuals or population groups to known carcinogens in their environment, 3) development of immunologic measures to inhibit or modify tumor growth, and their applicability in human subjects.

The staff of the Biology Branch is divided among four sections: Cytogenetics and Cytology, Cellular Immunity, Immunochemistry, and Tumor Antigen. Members of the Cytogenetics and Cytology Section have developed several cell transformation models suitable for the rapid screening of agents for carcinogenic potential. The practical value of these models is now being tested elsewhere. Studies in the Cellular Immunity Section on Mycobacterium bovis (strain BCG) have been helpful in the design of clinical studies in about 50 centers throughout the world to determine the value of BCG in the treatment of human cancer. Immunological interference with the carcinogenic process may also be accomplished with reagents of the humoral immune system, i.e., antibody and complement. Studies of humoral tumor immunity are being carried out in the Immunochemistry Section, while the major mission of the Tumor Antigen Section requires isolation and characterization of tumor-specific antigens.

Immunochemistry Section - *"Conducts studies into the immunochemistry of antibodies, antigens, and the components of complement. Elucidates the mechanisms of cytotoxic reactions. Develops and applies immunochemical tools to the detection and enumeration of normal and malignant antigens."*

In studying the mechanism of cytotoxic reactions it was discovered that two guinea pig hepatoma cell lines, L1 and L10, differ in their susceptibility to cytotoxicity by Forssman antibody or specific antitumor antibody and complement (C). Sensitized tumor cells were killed more efficiently with human complement than with guinea pig C. Antigen concentrations and/or class of antibody were not the sole reasons for these observations. The results suggest a deficiency in the fixation of hemolytically active C components to the cell surface.

To study the fixation of various C components to cells, immunochemical techniques were developed which could detect nanogram amounts of guinea pig and human C4 and C3 bound to the cell surfaces. The results indicated that hundreds of thousands of guinea pig and human C4 and C3 molecules were fixed onto the cells of two tumor lines. Therefore, the observed inefficiency of cytotoxicity induced by guinea pig C was not due to lack of fixation of C4 and C3. The defect may be at some later step in the C sequence or in the inefficient activation of the early components. Methods are being developed to study these questions.

In efforts to detect an immune response in guinea pigs to L1 and L10 cells in vivo, studies have been initiated to determine the amount of host immunoglobulin (presumed to be antitumor antibody), C4 and C3 molecules on fresh tumor cells harvested on different days after intraperitoneal implantation. Cell bound immunoglobulin (antibody) was detected using an indirect C1 fixation and transfer test employing a rabbit anti-guinea pig immunoglobulin (Ig) reagent. Guinea pig C4 and C3 are detected using the methods mentioned earlier. Preliminary results, with L10 cells, indicate that levels of bound C4 and C3 increased up to day 13 after implantation, then decreased on day 15 (the last day sampled). The amount of cell bound Ig did not change at the time intervals tested. The question of cell surface dynamics were also studied in vitro. Tumor cells stored at 4°C and in tissue culture at 37°C for up to two days appeared to lose C4 and C3. Decreased levels of Ig were detected on cells stored at 37°C. Tumor cell growth was not detected during this time period in culture. Further studies are in progress to verify these observations.

To obtain better sources of tumor antigens and to study the phenomenon of antigen shedding, exhausted tissue culture fluids from cultured guinea pig hepatoma cell lines (L10) and ascites fluid from L10 tumor-bearing guinea pigs were collected, ammonium sulfate precipitated, and concentrated 10-fold by dialysis.

The presence of tumor-specific antigens in the concentrate was demonstrated by double diffusion in gel, inhibition of fluorescent antibody, inhibition of cytotoxicity, and induction of delayed hypersensitivity reaction.

Double diffusion technique releaved that the antigen preparations yielded at least two lines of precipitation when reacted with specific rabbit anti-L10 antiserum. This finding implied the presence of two antigenic determinants specific for line-10 tumor. To determine antigen specificity the rabbit anti-L10 antiserum was absorbed with various materials. It was found that lyophilized guinea pig serum, L1 cells and normal guinea pig peritoneal cells did not remove anti-soluble antigen activity; absorption of the serum with intact L10 cells removed this activity.

In collaboration with the Tumor Antigen Section, the biological function of rabbit antibody against line-10 cells was studied. Rabbit antibody coated line-10 cells fixed not only guinea pig C1 but also whole complement. The antibody was cytotoxic with human, rabbit complement and under special conditions with guinea pig complement. Furthermore, spleen cells from unimmunized guinea pigs killed rabbit antibody-coated line-10 cells in the absence of added complement. Thus the in vitro tests indicated two mechanisms by which rabbit antibody could damage line-10 tumor cells: by activation of the complement system or by activation of non-immune lymphocytes. Studies with the xenogeneic antibody in vivo have shown that intradermally inoculated line-10 cells mixed with rabbit antibody grew poorly or not at all. It is of further interest that mixtures of tumor cells with specific guinea pig antibody (for which no biological activity in vitro was demonstrated) grew as well as control cells.

So far, all chemically induced guinea pig tumors were shown to contain TSA. Such antigens serve as convenient markers for the presence of tumor cells. It is not known, however, whether the appearance of such antigens always accompanies transformation. To study this question, a joint venture with members of the Cytogenetics and Cytology Section was initiated. Guinea pig embryonic fibroblasts can be transformed in vitro (see Cytogenetics and Cytology Section) by selected carcinogens. An untreated embryonic cell line and two cloned morphologically distinct lines developed by treatment of embryo cells with carcinogens of different classes were chosen for study. All three lines appear to share antigens and absorption tests suggest the transformed cells may possess specific antigens. Attempts to raise specific antisera with workable titers in guinea pigs and rabbits have met with limited success. Different routes and protocols for immunization, including presence and absence of adjuvant as well as treatment with neuraminidase, have been attempted. Techniques used in these studies include fluorescent microscopy, C1 fixation and transfer, complement lysis and colony inhibition tests.

Cellular Immunity Section - *"Plans and carries out investigations (1) to determine the types of host immune cells that are toxic to cancer cells; (2) to elucidate the process by which these host cells destroy tumor cells; (3) to find methods to increase the number of such cells in the host; and (4) to study possible interactions of carcinogens with host immune cells or with the apparatus responsible for the production of host immune cells."*

Studies were completed on ability of bacterial cell walls to cause tumor regression in guinea pigs. Effective bacterial preparations contained cell walls of Mycobacterium tuberculosis, Mycobacterium bovis or Mycobacterium kansasii attached to mineral oil droplets. Cells walls of M. smegmatis, Nocardia asteroides and Corynebacterium parvum lacked tumor suppressive properties. Treatment of BCG cell walls with proteolytic enzymes, organic solvents, acid or alkali decreased tumor suppressive properties.

Experiments were continued in mice to determine the basis for BCG mediated tumor suppression. The results of these experiments imply that a class of lymphocytes is essential for suppression of tumor cell growth at the site of BCG infection. Other studies in mice emphasize the importance of the macrophage for BCG mediated tumor regression. An in vitro assay was developed which demonstrates tumor cytotoxicity in vitro by macrophages from mice injected with BCG. Macrophages from BCG infected mice are more effective in killing a variety of neoplastic cells then in killing embryonic (normal) cells. Other immunopotentiators have been evaluated in mice. Infection with Listeria monocytogenes was shown to be effective in causing regression of a mouse fibrosarcoma. Administration of a synthetic polymer (pyran) was found to inhibit tumor growth and produces a population of macrophages with cytotoxic activities.

Attempts were made to develop additional animal models for immunotherapy of human cancer. Attempts to treat a guinea pig with metastatic disease restricted to one lymph node by systemic application of BCG were unsuccessful. Elimination of microscopic lymph node metastases required injection of BCG into the established intradermal tumor. Attempts to treat the Lewis lung tumor in mice with BCG were unsuccessful.

Investigations continue on the cellular basis for tumor graft rejection in guinea pigs. These investigations indicate that rejection of a tumor in an immunized host is accompanied by less injury to normal "bystander" cells than is rejection of a tumor in an unimmunized host.

A variety of investigations were designed to understand the role of the immune factors in the control of tumor growth in the large bowel. These studies indicated: 1) intracolonic injection of BCG in the rhesus monkey is not associated with morbidity or mortality; and 2) transplanted tumor growth can be suppressed at the site of BCG infection in guinea pig colons.

Studies of aryl hydrocarbon hydroxylase (AHH) indicated that enzyme activity was present in a variety of lymphoid tissues in guinea pigs and that it could be localized in peritoneal and alveolar macrophages, but not in lymph node lymphocytes. In collaboration with Dr. H. V. Gelboin and Dr. J. Whitlock of the Chemistry Branch and with Dr. P. Chretien of the Surgery Branch, we have attempted to develop an assay for AHH in human peripheral blood leukocytes. Very little enzyme activity could be detected in leukocyte rich plasma or in preparations of polymorphonuclear leukocytes treated in vitro with benz(a)anthracene. Higher levels were found in human peripheral blood lymphocytes that had been incubated with phytohemagglutinin (PHA) and 3-methylcholanthrene; enzyme activity appeared directly related to the concentration of PHA.

Tumor Antigen Section - *"Conducts a research program on tumor specific antigens in experimental animals and man: development of methods for antigen assay; extraction and purification of antigens for induction of immune defense against tumor cells and for production of antibodies that can be used as markers in carcinogenesis studies. Investigates problems in cell biology related to host response to tumor antigens: cell membrane antigen movement; mononuclear cell chemotaxis; isolation and mechanism of action of lymphotoxin."*

During the past year work in this Section has covered three areas: (1) purification of tumor antigen and immunogenicity of the products, (2) mechanisms of tumor cell killing, and (3) chemotaxis.

(1) Tumor Antigen - Tumor specific antigen extracted from guinea pig line-10 tumor with 3M KCl is heterogeneous with respect to size as determined by gel filtration, density as determined by sucrose density ultracentrifugation and precipitability by ammonium sulfate. The yield of antigen, assayed by skin test in a line-10-immune guinea pig, is 10-20%; and since the missing antigen cannot be accounted for in the extracted cell pellet, it is probably destroyed during the extraction procedure.

A method for obtaining line-10 cell membranes free of nuclei and the bulk of other intracellular constituents was devised. The method involves slow addition of sucrose to the cell suspension until the concentration is 17.5%, after which the cells are centrifuged and the cells in the pellet are ruptured osmotically by the addition of water. Nuclei and membranes can then be separated by a 1 minute centrifugation. This method is applicable to line-10 cells; the membranes of line-1 tumor, for example, are broken into multiple fragments at the osmotic shocking stage and this makes separation of membranes from intracellular organelles impossible with these simple techniques. With line-10 cells the method provides virtually intact membranes or large fragments in yields of 15-25%; the antigenic activity by skin test or antibody absorption tests is comparable to that of intact cells.

The capacity of KCl extracts and membranes to protect guinea pigs against tumor cell challenge was studied. Immunization with line-10 extract emulsified with Freund's adjuvant or mixed with Ribi vaccine led to delayed cutaneous hypersensitivity when animals were challenged with viable cells. All of the animals were not protected against tumor in contrast to 100% protection after immunization with viable cells. Comparing immunogenicity of whole cells, KCl extract and cell membranes mixed with Ribi vaccine, it was found that the membranes immunized poorly and did not protect against tumor challenge. This important finding poses the question whether the cell membrane tumor specific antigen was altered in subtle ways not detected by skin test or antibody absorption or whether immune processing of the cell membrane is different from that of the viable cells. In studying the humoral response of guinea pigs to line-10 cells, antibody was found in serum of guinea pigs immunized against line-10 tumor with a line-10 BCG vaccine, and also in animals with progressively growing and metastasizing tumors. No correlation could be made between antibody levels and the course of tumor growth in the host animal. No biological function such as C fixation nor lymphocyte cytotoxicity could be ascribed to the antibody.

(2) Mechanism of Tumor Cell Killing - The non-specific destruction of target cells that may occur at the site of a cellular immune reaction was studied in collaboration with the Cellular Immunity Section. Peritoneal cells from BCG immune guinea pigs were stimulated by the addition of purified protein derivative of tuberculin. When line-10 tumor cells were added to this mixture, a significant proportion of the tumor cells were destroyed during the ensuing 48 hours. To determine the cell type causing the cytotoxicity, the peritoneal cells were separated into a non-adherent or lymphocyte-rich fraction and an adherent fraction comprised almost entirely of macrophages. The cytotoxic action of the purified protein derivative of tuberculin stimulated BCG immune peritoneal cells was accounted for by the lymphocyte-rich population and no cytotoxic role could be assigned to macrophages. This result is in contrast to histological findings that histiocytes may play a role in the in vivo destruction of tumor cells.

The role of activated macrophages was studied in collaboration with the Cellular Immunity Section and the Laboratory of Biochemistry, (DCBD, NCI). Macrophages from BCG-infected mice (activated macrophages) induce localized areas of tumor cell monolayer destruction in vitro (plaque formation). Plaque formation on tumor cell monolayers appears to be the end result of both cytostasis and cytolysis. The macrophage action is immunologically non-specific and can be shown with syngeneic, allogeneic and xenogeneic tumor target cells. Tumor monolayer destruction requires intimate contact between macrophages and tumor cells for 1-2 days and does not involve phagocytosis. Soluble cytotoxins have not been found in the macrophage-tumor cell culture supernatant fluids. Although the reaction is immunologically non-specific, normal embryo cells (syngeneic, allogeneic and xenogeneic) are quantitatively less susceptible to macrophage action than are tumor cells. The basis for this differential susceptibility, which was previously shown in this laboratory for lymphotoxin, is currently under investigation.

(3) Chemotaxis - During the past year the scope of studies on chemotaxis was extended, since the in vitro method allows a quantitative look at both production of chemotactic lymphokines and the response of the cells. Studies on the monocyte chemotactic response in cancer patients were completed, showing that many cancer patients had defective monocyte chemotactic responses (MCR's), sometimes at early stages of disease. Low MCR's were found in patients with malignant melanoma, squamous carcinoma, lymphoma, sarcoma and adenocarcinoma. Local infection in some patients were thought to abrogate this defect temporarily. Retrospective analysis of melanoma patients indicated that those with normal MCR's developed metastases more slowly than those with low MCR's. These studies identified a defect in cancer patients in the effector arm of the immune response which may relate to their failure to reject a tumor. Followup of the initial study group of patients is continuing, to relate tests results to ultimate prognosis. Further study of this abnormality with regard to prognosis and treatment is being done under contract. We have also begun studies to determine the mechanism of low MCR's in cancer patients, beginning with a search for serum factors which could inhibit the response of normal monocytes in vitro.

Because basophils constitute part of the antigen-specific antisera reaction, the migration of human basophils has been observed and quantitated in a new system for the first time. A new lymphokine was discovered which increases the response of human basophils to subsequent testing with an unrelated chemotactic stimulus derived from C5a. Studies with this factor may increase knowledge about states of delayed hypersensitivity involving basophil infiltrates and the role of basophils in tumor immunity.

Cytogenetics and Cytology Section - *"Studies carcinogenesis in mammalian cells. The effects of various carcinogens are studied both in vitro and in vivo employing models designed primarily to investigate somatic cell variation. The systems in use include cell and organ cultures, and intact animals, bridging the biological and chemical approaches to carcinogenesis. Biological phenomena (carcinogenesis or differentiation) are correlated with alterations of nucleic acids and proteins through biochemical, cytogenetic, immunological and mutagenic investigations. Projects under way deal with: (1) the in vitro effects of biological, chemical and physical carcinogens including assessment of chromosomal alterations, mutagenic properties and identification of factors modifying the development of the carcinogenic process; (2) host-mediated in vivo - in vitro carcinogenesis; (3) identification of cell surface and other cellular alterations associated with the transition from the growth-controlled to the neoplastic state; and (4) study of potential environmental carcinogenesis in cells in culture and in intact animals."*

As part of the program to determine the factors that influence chemical transformation, studies dealing with the interaction of potent chemical carcinogens with either weak chemical carcinogens or physical agents has been continued. The previous report that pretreatment of hamster cells with X-irradiation prior to treatment with a potent chemical carcinogen enhances transformation has been extended to the point where it is now possible to state that the kinetics of transformation are identical to those obtained without pretreatment with X-irradiation. With a constant dose of irradiation,

250 R, and varying concentrations of benzo(a)pyrene the dose response curve is consistent with a one-hit phenomenon. Pretreatment of cells for 1 hr with the alkylating agents ethyl methanesulfonate or methyl methanesulfonate prior to treatment with a variety of known chemical carcinogens results again in a maximum enhancement occurring when the potent carcinogen is added 48 hrs subsequent to the addition of the alkylating agent. This enhancement is constant whether or not the cells are treated in mass culture or after seeding for colony formation. The results differ from those obtained with irradiation in two ways: a) no enhancement occurs at time intervals prior or subsequent to 48 hrs, whereas with the X-irradiation there was a gradual increase in the degree of enhancement with time to 48 hrs; b) pretreatment with alkylating agents results in enhancement ratios slightly lower than those produced by X-irradiation treatment. Enhancement by an alkylating agent could not be explained on the basis of changes in cell cycle or the formation of single-stranded breaks since there was no alteration in normal cell DNA synthesis through 48 hr post-treatment. It must be concluded that the repair of DNA as it is now understood is probably not involved. The possibility that enhancement is due to some type of cross-feeding or artifact is remote. Ultraviolet light used for the primary treatment, gave no enhancement of benzo(a)pyrene transformation. Also, cells treated with caffeine subsequent to treatment with a number of different carcinogens showed the maximum enhancement as early as 4 hrs after carcinogen treatment. The addition of caffeine at 24 or 48 hrs post-potent carcinogen treatment does not increase the enhancement.

In the past, this laboratory reported that hamster cells, mouse cells and guinea pig cells were transformed with chemical carcinogens. It is now reported that it is also possible to transform rat embryo cells. Although others have reported that mass cultures of a continuous rat line may be transformed by carcinogenic polycyclic aromatic hydrocarbons or that a virally infected line may be transformed by chemical carcinogens, this is the first report of the transformation phenomenon in random-bred, germ-free and specific-pathogen-free rats. It was possible to add carcinogen to secondary subcultures and to recognize the transformation at the next subculture. These experiments were designed to compare the chromosomal variability of control cells, transformed cells, and non-transformed cells isolated from carcinogen-treated dishes with the chromosomes of cells obtained as a result of injecting subcutaneously a carcinogen into male rats.

Chromosomes of cells transformed in vitro were also compared with chromosomes of in vitro transformed cells implanted and grown in vivo. The results of these comparative studies indicated that neither in vitro transformation nor in vivo tumor induction is accompanied by a specific numerical or structural chromosomal aberration.

Hamster chromosomes differ from those of other species in that they have relatively large amounts of constitutive heterochromatin. Since variation in the amount of constitutive heterochromatin per cell has been considered responsible for changes in phenotypic effects referred to as position effect, transformed hamster lines and tumors derived from these were examined in terms of constitutive heterochromatin. Syrian hamster cells transformed by

chemical carcinogens have chromosomes with constitutive heterochromatin characteristic of the species. Heterochromatin alterations occurred in only two different tumor cultures and in no transformed line and thus cannot be considered a causative factor in transformation.

The host-mediated in vivo-in vitro transformation system reported last year is being utilized in an attempt to determine the number of different types of cells that can be transformed from a specific organ and for the purpose of determining if it is possible to obtain transformed epithelial-like cells from a specific organ. As anticipated, it was possible to obtain variation in clone morphology of chemical carcinogen transformed hamster cells. Currently, efforts are directed to obtain carcinomas from colonies that have epithelial-like morphologies.

The question concerning the significance of chromosome changes associated with transformation, reversion or variants has been considered using transformed Balb/3T3 lines. The relationship between major changes in chromosome number and the behavior of the cells is not as clear as implied in some of the literature. Chromosome analysis of the variant lines demonstrated that some variants may have an increased or a decreased number of chromosomes compared to the parental transformed cell lines. Although chromosomes of mouse lines are characterized by their similarities, all variants had unique marker chromosomes. The distribution of marker chromosomes and heterochromatin suggest that the initial event in variant formation was a reduction in chromosome number with a subsequent polyploidization of the reduced chromosome complement.

Attempts to transform human fetal cells have been frustrating. When known chemical carcinogens were used, morphological transformation and toxicity often did not occur or were of transitory nature. In some instances, it appeared that permanently transformed lines had been obtained but in no case has it been possible to demonstrate tumor production in a xenogenic host.

Because of the partial overlap of the properties of carcinogenicity and mutagenicity, experiments are underway to determine the extent to which these two phenomena occur in mammalian cells used in this laboratory. Thus, experiments in mutagenesis are performed in consort with those dealing with in vitro carcinogenesis. In order to maximize mutation frequencies observed after treatment with carcinogenic chemicals, many of which are toxic, it has been necessary to study a number of factors that may influence the observed frequency of mutations. Using a new method which permits cells to recover for several days it is now possible to study the effects of more than one agent and to determine if they result in the enhancement of azaguanine resistant mutant cells.

Thus far, 20 cell strains derived from freshly isolated diploid guinea pig cells exposed in utero or after introduction into culture to a chemical carcinogen or non-carcinogen have been evaluated for a number of different biological characteristics. The acquisition of growth properties and morphological alterations obtained with guinea pig cells is similar to those seen with transformation of mouse, rat, and hamster cells; however,

the characteristics do not occur coincidently with the appearance of tumor growth. Some of the indices of neoplastic transformation are observed prior to the attainment of progressive tumor growth. The guinea pig model, as other animal species, responds to carcinogens from different chemical classes, but thus far spontaneous transformation has not been observed. The primary steps leading to neoplasia in guinea pig cells are probably identical to those in other animal species; however, the temporal relationship suggests that the events are not telescoped as they are in the mouse, rat and hamster. The apparent extended sequence of events in guinea pig cells during the transition to neoplasia provides avenues for the study of distinct alterations shared presumably by all mammalian cells in the development of cancer.

Serial No. NCI-4664
1. Biology Branch, Carcinogenesis, DCCP
2. Cellular Immunity Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Immunologic Approaches to the Prevention and Treatment of Cancer

Previous Serial Number: Same

Principal Investigators: Berton Zbar, M.D. and Herbert J. Rapp, Sc.D.

Other Investigators: Robert C. Bast Jr., M.D., Richard Harmel, M.D., Adam Bekierkunst, Ph.D.

Cooperating Units: For supply of BCG and studies of different BCG preparations: Trudeau Institute, Saranac Lake, New York. For studies with nonliving mycobacterial preparations: Dr. Edgar Ribi, Rocky Mountain Laboratory, Hamilton, Montana. For studies of metastases: Howard Smith, M.D., Tumor Antigen Section, Biology Branch, NCI. For studies of BCG and colon cancer: Norman Zamcheck, M.D., Harvard Medical School, Boston, Massachusetts; Dan Delgard, M.D., Hazelton Laboratories, Hazelton, Virginia; Jerry Ward, M.D., Experimental Pathology Branch, NCI.

Man Years:

Total:	3.0
Professional:	2.0
Other:	1.0

Project Description

Objectives:

The primary objective of this project is to establish conditions and methods suited to the prevention and treatment of cancer. This objective is based on the finding that tumors contain unique antigens capable of eliciting the formation of tumor specific humoral antibodies and of causing the appearance of cells which react specifically with tumor antigens. Transplantable syngeneic guinea pig tumors will be used to investigate conditions for effective immunotherapy.

Methods Employed:

Tumors. Malignant hepatomas have been induced in strain-2 guinea pigs by

feeding them diethylnitrosamine in their drinking water. Transplantable ascites tumors have been derived from primary hepatomas. Two of these ascites variants are being maintained by periodic intraperitoneal transplantation. Portions of these tumors are periodically frozen and stored in liquid nitrogen.

Studies on effect of method of preparation on tumor suppressive properties of BCG, nonliving mycobacterial preparations and treatment of microscopic lymph node metastases were carried out with cells of a transplantable hepatoma (line-10). This tumor when injected into the skin produces a progressively growing papule and regional lymph node metastases within a few days.

Tumor Suppressive Properties of Mycobacterial Preparations. Tumor cells were injected intradermally into unimmunized strain-2 guinea pigs. When established intradermal tumors and regional lymph node metastases were present (7 days after intradermal injection) BCG preparations were injected into the tumor.

Effect of Method of Preparation on Tumor Suppressive Properties of BCG. Freeze dried preparations of the Tice substrain of BCG were obtained from the Tuberculosis Research Laboratory of the University of Illinois. BCG cultivated in liquid medium in the absence of a detergent grew as a pellicle on the surface of the medium. Pellicles were ground in a mechanical mill; the dispersed preapration was frozen and dried. About 5 percent of the bacteria in these preparations were viable.

Fresh frozen preparations of the Tice substrain of BCG were prepared at the Trudeau Institute. BCG cultivated in the presence of a detergent grew below the surface of the medium as a dispersed culture. The bacteria in culture medium were frozen. About 60-90% of bacteria in these preparations were viable.

Nonliving Mycobacterial Preparations. Bacterial cell walls and cell wall extracts were prepared in the laboratory of Dr. Edgar Ribi. Cell walls or cell wall extracts were mixed with a small amount of mineral oil and this mixture was ground to a smooth paste. Saline containing tween 80 was added to the paste and grinding was continued until a well dispersed oil in water emulsion was obtained.

Microscopic Lymph Node Metastases. One million line-10 cells were injected intradermally into strain-2 male guinea pigs. Seven days after inoculation, intradermal tumors were excised. Guinea pigs treated in this manner were free of intradermal tumors but had 10^5 tumor cells in the superficial distal axillary lymph node.

Major Findings:

Effect of Method of Preparation on Tumor Suppressive Properties of BCG. The

tumor regressive properties of freeze dried Tice BCG and fresh frozen Tice BCG were compared. Preparations were adjusted to contain the same concentration of viable organisms. Fresh frozen BCG caused regression in 77% treated animals; freeze dried BCG caused regression in 26% of treated animals.

Nonliving Mycobacterial Preparations. Studies were performed to identify components of nonliving mycobacterial preparation essential for tumor regression.

1. Effective preparations contained BCG cell walls attached to mineral oil droplets. Preparations containing BCG cell walls attached to vitamin A droplets or pristane had minimal or no antitumor effect.

2. Deproteinized, delipidated BCG cell walls lacked tumor regressive properties. Addition of a trehalose-mycolic acid ester (P3) to the deproteinized, delipidated BCG cell walls restored tumor regressive properties.

3. P3 could not resotre tumor regressive properties of deproteinized, delipidated BCG cell walls completely devoid of protein.

Treatment of Microscopic Lymph Node Metastases.

1. Excision of intradermal tumors infected with BCG was effective in preventing the growth of microscopic lymph node metastases.

2. Therapy was effective when tumors were excised 24 hours or 168 hours after the initiation of BCG infection. There was a suggestion that therapy might be effective when tumors were excised 15 minutes after initiation of BCG infection.

3. Fifteen minutes after intratumor infection, 10^5 BCG were present in the superficial distal axillary lymph node; about 10^4 BCG were present in the high axillary lymph node.

4. Several other routes of BCG administration were tested for ability to eradicate microscopic lymph node metastases. BCG administered by the following routes failed to eradicate metastases: intranodal, footpad, flank (4 sites), flank (scarification).

Attempts to Treat Tumors in the Colon with BCG.

1. Injection of BCG into the colon of the rhesus monkey was not associated with significant morbidity or mortality.

2. Injection of BCG into the rectal section of the colon of the rhesus monkey led to the development of a chronic inflammatory reaction at the site of infection. Chronic inflammatory reactions were not produced in the sigmoid section of the colon by BCG injection.

3. Tumor cells (well-differentiated hepatocarcinoma) were injected into the sigmoid colon of inbred syngeneic guinea pigs. Guinea pigs died 25-30 days after tumor implantation.

4. BCG infected in admixture with tumor cells into the sigmoid colon suppressed tumor growth.

5. The growth of a rat transplantable colon tumor was suppressed when injected in admixture with BCG cell walls attached to oil droplets.

Significance to Biomedical Research and the Program of the Institute:

BCG preparations are potent inhibitors of tumor cell growth. Although this observation has been repeatedly made, it has been difficult to harness the tumor suppressive properties of BCG. The results presented suggest two conditions necessary for optimal use of BCG. BCG preparations should be prepared in the presence of a detergent and then stored as frozen cultures. This method of preparation ensures retension of tumor suppressive properties of BCG. BCG preparations should be injected into tumors; infected tumors may then be excised. This procedure ensures maximal tumor suppressive effect.

Nonliving mycobacterial preparations are potent inhibitors of tumor growth. Such preparations avoid the risk inherent in the use of living bacteria. The results indicate that one component of the mycobacterial cell wall essential for tumor suppression has been identified.

Proposed Course of Project:

We will continue to perform experiments designed to determine the BCG substrain with maximal tumor suppressive activity and to determine conditions for preservation of antitumor activity. We will pursue attempts at isolating the molecules responsible for the tumor suppressive properties of BCG cell walls.

We will perform experiments to determine the optimal times for excision of intradermal tumors infected with BCG. We will attempt to develop models for impairing the gorwth of colon and breast tumors.

Publications

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Serial No. NCI-4665
1. Biology Branch, Carcinogenesis, DCCP
2. Cellular Immunity Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mechanism of Delayed Hypersensitivity and Tumor Graft Rejection

Previous Serial Number: Same

Principal Investigators: Berton Zbar, M.D., and Herbert J. Rapp, Sc.D.

Other Investigators: Robert C. Bast Jr., M.D., Ronald P. Cleveland, B.A., Richard Harmel, M.D.

Cooperating Units: For studies of macrophage mediated cytotoxicity: Monte S. Meltzer, M.D., Tumor Antigen Section, Biology Branch, NCI. For supply of Listeria monocytogenes: Trudeau Institute, Saranac Lake, New York.

Man Years:

Total:	2.2
Professional:	1.2
Other:	1.0

Project Description

Objectives:

The main objectives of this project are: 1) to define the mechanism of the immunologic rejection of antigenic tumors and 2) to determine the basis for suppression of tumor growth at the site of bacterial infections.

Methods Employed:

Guinea Pig Tumors. Malignant hepatomas have been induced in strain-2 guinea pigs by feeding them diethylnitrosamine in their drinking water. Transplantable ascites and intramuscular variants of these tumors currently maintained in this laboratory are used.

Mouse Tumors. A variety of mouse sarcomas of the C3H strain are available. Portions of the tumors are frozen and stored in liquid nitrogen. They are maintained by serial transplantation in syngeneic mice which have been immunosuppressed by thymectomy and 450 r of x-irradiation.

Tumor Cell Mixing Experiments (Guinea Pig). Mixtures of line-1 and line-10

cells were prepared and injected intradermally into strain-2 male guinea pigs. Unimmunized strain-2 male guinea pigs were recipients in some experiments. Guinea pigs immunized to line-1 cells were recipients in other experiments. Measurements of intradermal tumor nodules and regional lymph nodes were made weekly.

Suppression of Tumor Growth at the Site of Infection with Listeria Monocytogenes. Mouse sarcomas were minced and dispersed enzymatically with Pronase and DNAase. Appropriately diluted suspensions of tumor cells are injected intradermally into syngeneic mice either alone or after mixture and short incubation with living Listeria monocytogenes. In other cases, living Listeria monocytogenes is infiltrated into tumors which have been growing in mice for several days. Tumors are measured weekly.

Guinea pig ascites tumor cells are injected intradermally into syngeneic guinea pigs either alone or after mixture and short incubation with living Listeria monocytogenes. In some experiments established intradermal tumors in guinea pigs are treated by intralesional injection of living Listeria monocytogenes.

In Vitro Assay of Macrophage Mediated Cytotoxicity. Peritoneal cells were obtained from mice injected by the intraperitoneal route with living BCG, pyran copolymer or diluent. Peritoneal cells (2×10^6) were placed in the center of 35 mm Falcon plastic dishes. Nonadherent peritoneal cells were removed by irrigation of the petri dishes with tissue culture medium. Target cells (tumor cells, or embryonic cells) were added to the petri dish containing adherent peritoneal cells; dishes were incubated for 72 hours at $37^{o}C$. Cultures were examined daily under the phase microscope; petri dishes were dried and stained at 72 hours. Target cell injury was graded as follows: 1) 0: minimal or no target cell injury. Target cell monolayers in the macrophage-containing areas (center) of the plate were intact and at a cell density similar to cells in the periphery or to cells in cultures without macrophages; 2) +/-: target cell injury apparent but incomplete. Areas of intact target cell monolayers present in the center of the plate but at a lower cell density than cells in the periphery of cells in cultures without macrophages; 3) +: complete destruction of the target cell monolayer in the center of the plate.

Studies with Pyran Copolymer. Mice were treated with five daily intraperitoneal injections of pyran copolymer (25 mgm/kg). In some experiments peritoneal cells were collected seven days after the last injection of pyran; in other experiments tumor cells were injected intradermally seven days after the last injection of pyran.

Major Findings:

Basis of Delayed Hypersensitivity and Tumor Graft Rejection.

1. Intradermal injection of cells of a transplantable hepatoma (line-1) into normal syngeneic guinea pigs led to the formation of a tumor which regressed.

2. Intradermal injection of cells of a different transplantable hepatoma (line-10) into normal syngeneic guinea pigs led to the formation of an intradermal tumor which grew progressively, and lymph node metastases.

3. When a mixture of line-1 and line-10 tumor cells were injected intradermally into normal syngeneic guinea pigs a tumor formed which regressed. The growth of line-10 tumor cells was inhibited at the site of rejection of line-1 tumor cells.

4. When line-1 and line-10 tumor cells were injected intradermally at separate sites in normal syngeneic guinea pigs: line-1 cells grew and regressed, line-10 cells grew progressively.

5. Inhibition of line-10 tumor cell growth required: a) close contact between line-10 cells and line-1 cells; b) an adequate dose of line-1 cells; c) line-1 cells capable of dividing; d) an appropriate route of inoculation.

6. The growth of line-10 tumor cells was not inhibited at the site of rejection of line-1 tumor cells in guinea pigs immunized to line-1 tumor.

Tumor Suppression at the Site of Infection with Listeria Monocytogenes (LM).

1. Growth of a mouse fibrosarcoma and of a guinea pig hepatoma was suppressed completely when tumor cells were mixed with viable LM prior to intradermal injection into syngeneic recipients.

2. Regression of established mouse fibrosarcoma and guinea pig hepatoma transplants was produced in a fraction of animals by intratumor injection of LM.

3. Injection of murine tumor cells and LM at separate sites did not effect tumor growth.

4. Heat killed LM could produce suppression of mouse fibrosarcomas if 100 times more organisms were mixed with tumor cells prior to injection.

5. Intradermal injection of 10^6 LM killed a significant fraction of mice, while 10^9 LM were required to kill guinea pigs.

Tumor Cytotoxicity by Macrophages from Mice Infected with Mycobacterium bovis Strain BCG.

1. Peritoneal macrophages from mice infected with BCG by the intraperitoneal route were cytotoxic for tumor cells. Peritoneal macrophages obtained from mice infected with BCG by the intradermal route were not cytotoxic for tumor cells. Cytotoxic macrophages were obtained three days after initiation of infection by the intraperitoneal route.

2. Macrophage mediated cytotoxicity required: close contact between macrophages and tumor cells, an adequate number of effector macrophages. Macrophages were cytotoxic for proliferating or nonproliferating tumor cells.

3. Tumor cytotoxicity was observed 48 hours after initiating in vitro cultures of macrophages and tumor cells.

4. Macrophages from mice infected with BCG killed syngeneic, allogeneic and xenogeneic tumor cell lines.

5. Macrophages from mice not infected with BCG, or infected with BCG by the intradermal route did not kill tumor cells or normal embryonic cells.

6. Tumor cells were more susceptible to macrophage mediated cytotoxicity than embryonic cells.

Tumor Suppression in Mice by Pyran Copolymer.

1. Pyran administered by the intraperitoneal route suppressed the growth of a subsequent intradermal injection of a transplantable fibrosarcoma. The latent period was prolonged in pyran treated mice compared to mice treated with diluent.

2. Peritoneal cells from mice treated with pyran by the IP route were cytotoxic for tumor cells in vitro, and suppressed the growth of tumor cells in vivo.

3. Pyran given by the subcutaneous or intravenous route did not produce populations of cytotoxic macrophages.

Production of Cytotoxic Macrophages In Vitro.

1. Spleen cells were obtained from normal syngeneic mice cultured for three days in tissue culture medium containing mouse serum. Macrophages obtained from normal syngeneic mice cultured for three days in the medium conditioned as described were cytotoxic for tumor cells.

Significance to Biomedical Research and the Program of the Institute:

The cellular and molecular basis for tumor graft rejection remain unknown. Studies presented in this report indicate that rejection of tumor grafts is different in unimmunized compared to immunized animals. In unimmunized animals, rejection of antigenic tumor grafts is inefficient, i.e. tumor cell destruction is accompanied by destruction of antigenically unrelated cells (normal cells or antigenically unrelated tumor cells). In immunized animals, rejection of antigenic tumor grafts is efficient, i.e. tumor cell destruction is not accompanied by destruction of antigenically different cells. The cellular basis for graft rejection may differ depending on the immune state of the host.

The results of studies with Listeria monocytogenes confirm and extend the work of Hibbs and associates and Youdim. Listeria monocytogenes was more effective than BCG in suppressing tumor growth in mice. The converse was

true in guinea pigs. These puzzling observations may provide a key to improved understanding of the basis of antitumor activity of certain bacterial infection.

The development of a reproducible in vitro assay for macrophage mediated tumor cell killing opens several lines of investigation. What is the basis of macrophage mediated cell killing? What properties of malignant cells account for their heightened susceptibility to macrophage killing? Can cytotoxic macrophages be used to detect cells converted in vitro to the malignant state? Does the development of cytotoxic macrophages in the host correlate with the development of resistance to tumor growth?

Proposed Course of Project:

Preliminary experiments suggest that line-10 tumor cell growth is inhibited at the site of rejection of an allogeneic guinea pig fibrosarcoma. We will attempt to confirm this observation and compare the efficiency of suppression of line-10 growth in guinea pigs immunized to the allogeneic fibrosarcoma and in unimmunized guinea pigs.

We will determine optimal conditions for regression of established tumors with Listeria monocytogenes. Experiments will be performed to determine whether mixtures of living BCG and living Listeria monocytogenes act in a synergistic manner to eradicate tumor growth.

Attempts will be made to determine the basis of macrophage mediated cytotoxicity. To date, attempts to inhibit in vitro macrophage cytotoxicity have been successful. We will concentrate effort on converting normal macrophages to cytotoxic macrophages in vitro.

We will determine whether pyran has an antitumor effect in immunosuppressed mice. Attempts will be made to correlate the development of a population of cytotoxic macrophages with the development of resistance to tumor growth.

Publications

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Serial No. NCI-4679
1. Biology Branch, Carcinogenesis, DCCP
2. Cellular Immunity Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Interaction of Carcinogenic Polycyclic Hydrocarbons with Mammalian Lymphoid Tissue

Previous Serial Number: Same

Principal Investigators: Robert C. Bast, Jr., M.D., Harry V. Gelboin, Ph.D., Herbert J. Rapp, Sc.D.

Other Investigators: James P. Whitlock, M.D., Paul Chretien, M.D.

Cooperating Units: Chemistry Branch, OASDC, DCCP, NCI. Surgery Branch, DCBD, NCI.

Man Years:

Total:	0.6
Professional:	0.2
Other:	0.4

Project Description

Objectives:

The main objectives of this project are: 1) to measure the effect of polycyclic hydrocarbons on the microsomal and lysosomal enzymes of macrophages and lymphocytes; 2) to determine whether macrophages are capable of uptake, metabolism and binding of carcinogenic polycyclic hydrocarbons; 3) to find whether interaction of polycyclic hydrocarbons with macrophages facilitates the induction and/or expression of contact hypersensitivity to these compounds; 4) to develop a dependable, convenient *in vitro* assay for aryl-hydrocarbon hydroxylase (AHH) in human peripheral blood leukocytes.

Methods Employed:

Peritoneal macrophages were obtained from the abdominal cavities of guinea pigs, either without prior treatment or three days following the intraperitoneal injection of sterile mineral oil. Alveolar macrophages were isolated from mechanically dissociated guinea pig lungs.

AHH Activity of Macrophages in Cell Culture. To study the effect of benz(a)-anthracene (BA) on microsomal and lysosomal enzymes of macrophages in culture, peritoneal or alveolar cells were cultured in the presence or absence of BA. Adherence to culture dishes permitted separation of a macrophage-rich subpopulation that was washed, homogenized and assayed for AHH, B glucuronidase, acid phosphatase and lactate dehydrogenase.

Uptake and Binding of 7,12 dimethylbenzanthracene (DMBA). Peritoneal macrophages from animals treated with mineral oil +/- BA were cultured with tritium-labelled DMBA in the presence or absence of the AHH-inhibitor benzoflavone (BF). Following incubation cells were washed, homogenized and the total amount of intracellular DMBA was determined by scintillation counting. Protein in the macrophage homogenate was precipitated with trichloracetic acid (TCA) and the TCA precipitate was extracted exhaustively to remove contaminating lipid and nucleic acid. The amount of DMBA bound to protein was determined by scintillation counting.

Induction and Elicitation of Contact Hypersensitivity to DMBA. DMBA in acetone was applied to the shaved flank of strain-13 guinea pigs. After 1-2 weeks animals were rechallenged with multiple graded doses of DMBA in acetone on the contralateral flank. Cutaneous reactions were observed at 24 and 48 hours. For studies of inhibition BF was administered intraperitoneally in mineral oil or applied locally in acetone with the sensitizing or eliciting doses of DMBA.

Human Leukocyte Culture. Leukocyte-rich plasma was obtained by dextran sedimentation of heparinized human blood; granulocytes were isolated by adherence to plastic; lymphocytes were purified by standard column techniques. Leukocyte-rich plasma and granulocyte-rich fractions were incubated for 18 hours in the presence or absence of methylcholanthrene (MCA) and assayed for AHH. Lymphocytes were cultured with different concentrations of phytohemagglutinin (PHA) for 72 hours and treated with MCA for an additional 24 hours. Cells were assayed for AHH at the conclusion of incubation. Incorporation of tritiated thymidine was studied in parallel cultures.

Major Findings:

1. Treatment of alveolar and peritoneal macrophages with BA in cell culture increases the specific activity of AHH.

2. Levels of the lysosomal enzyme B glucuronidase and acid phosphatase are not affected by similar treatment with BA.

3. Covalent binding of DMBA to macrophage protein is not increased by pretreatment of cells with BA *in vivo*, and binding is not decreased in the presence of BF *in vitro*.

4. Local application or systemic administration of BF does not inhibit the development or expression of contact hypersensitivity to DMBA.

5. Very little AHH can be detected in human leukocyte-rich plasma or in purified granulocyte preparations. Greater activity is associated with purified lymphocytes that have been treated with PHA and MCA in cell culture. AHH levels vary with PHA concentration.

Significance to Biomedical Research and the Program of the Institute.

1. The microsomal mixed function oxygenase complex of mammalian cells metabolizes a variety of carcinogenic polycyclic hydrocarbons and facilitates their binding to cellular protein and DNA. AHH, a part of this complex, detoxifies several polycyclic hydrocarbons, but can also catalyze their conversion to more carcinogenic forms. Detection of AHH in macrophages and in lymphoid tissues, suggests that the reticuloendothelial system (RES) can participate in the metabolism of carcinogens. Macrophages contribute to inflammatory exudates throughout the body and could concentrate AHH at sites of chronic inflammation. Stimulation or depression of reticuloendothelial function alters the activity of a number of carcinogens. While these effects have generally been attributed to modulation of immune surveilance, the functional state of the RES might also influence the metabolism of carcinogens.

2. Studies of AHH in humans have been severely limited by the lack of a convenient assay for the enzyme in a readily available tissue. Newly developed assays depend upon the transformation of human peripheral blood lymphocytes into lymphoblasts in the presence of PHA or pokeweek mitogen. PHA responsiveness can vary dramatically in different disease states and the ability of experimental animals to respond to PHA is determined genetically. Consequently it is important to determine whether PHA treatment simply permits the expression of AHH activity or whether AHH levels are determined directly by the amount of lymphoblast transformation in response to the mitogen. Development of an assay that does not depend upon mitogen treatment would avoid these potential difficulties.

Proposed Course of Project:

1. To study the effect of BCG on AHH levels in guinea pig lymphoid tissue.

2. To explore the possible relationship between AHH and thymidine incorporation in human peripheral blood lymphocyte preparations that have been treated with PHA.

3. To measure AHH levels in human peripheral blood monocytes.

Publications

Bast, R.C., Jr., Shears, B.W., Rapp, H.J., Gelboin, H.V.: Aryl hydrocarbon (benz(a)pyrene) hydroxylase in guinea pig peritoneal macrophages: Benz(a)-anthracene induced increase of enzyme activity *in vivo* and in cell culture. J. Natl. Cancer Inst. 51: 675-678, 1973.

Serial No. NCI-4639
1. Biology Branch, Carcinogenesis, DCCP
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mechanism of Complement Fixation and Action

Previous Serial Number: Same

Principal Investigator: Tibor Borsos, Sc.D.

Other Investigators: Herbert J. Rapp, Sc.D. and Barbara D. Hooks, Ph.D.

Cooperating Units: None

Man Years:

Total	:	2.0
Professional	:	1.3
Other	:	0.7

Project Description

Objectives:

To apply rational complement fixation tests based on the fixation and transfer of the first component of complement to the analysis of antigen-antibody reactions, in particular in the search for cancer specific antigens.

Methods Employed:

The model for studying cytotoxic reactions mediated by antibody and complement consists of sheep erythrocytes, hemolytic antibody and guinea pig complement. Purification procedures for antibodies and the complement components include: preparative (large scale) gel filtration, ion exchange chromatography and preparative free electrophoresis. Other techniques used include precipitin and immunoelectrophoretic analysis, analytical, zonal and preparative ultracentrifugation and other immuno- and physico-chemical methods.

Major Findings:

The ultimate goal of this work is the development of immunological tools that permit exploration of (1) immunological approaches to cancer diagnosis and (2) possible host immune defense mechanisms against cancer. During the past year we have discovered that guinea pigs that were injected with tumor cells developed complement fixing antibodies to the tumor antigen. The antibody, although it fixes C1, does not seem to be cytotoxic. A very exciting

discovery was then made when it was found that control guinea pigs that were injected with BCG only also developed C1 fixing antibody to L10 tumor cells. We were able to show that the cross-reacting between BCG and L10 cells could be absorbed from the serum with L10 cells. We also found that BCG immune sera did not react with L1 tumor cells, implying that L10 and L1 did not share anti-BCG reactive antigen. This finding is a first report on cross-reactivity between a tumor cell antigen and a bacteria which is used in treatment of cancer.

In another series of studies we looked for the presence of guinea pig tumor-specific antibodies in the sera of normal (uninjected) rabbits. Current theories of antibody formation would predict the presence of low level of antitumor antibody in the sera of xenogeneic animals. With the use of the C1 fixation and transfer test we were able to detect a few ng/ml of anti-L10 and anti-L1 activity in the sera of normal rabbits. This finding is now followed up to determine how widespread in nature is the presence of preformed antitumor antibodies.

Significance to Biomedical Research and the Program of the Institute:

Complement fixation is one of the most widely used diagnostic tools. The development and successful application of a radically new and very sensitive complement fixation test, the C1FT test, opened up new possibilities in determining antibody-antigen reactions on cell surfaces.

Furthermore, cytotoxic reactions due to antibody and complement are prime examples of body defense mechanisms. We believe that fundamental research into the nature and mechanism of complement fixation and action will contribute greatly to the development of diagnostic tools and to the understanding of the mechanism of immune body defenses.

Proposed Course of Project:

This is a long-range project, and we expect little change in the scope of the work during the next few years. The ultimate goals of these projects are the development of better diagnostic tools based on fixation of complement and the elucidation of molecular events associated with the action of complement and antibodies. It is hoped that as a result of our program of inquiry into the basic problem of the interaction of antibodies, antigens and components of complement, tools will be developed that are of practical significance in our search for cancer antigens.

Publications

Borsos, T. and Rapp, H. J.: Brief communication: Antigenic relationship between Mycobacterium bovis (BCG) and a guinea pig hepatoma. J. Natl. Cancer Inst. 51: 1085-1086, 1973.

Borsos, T. and Rapp, H. J. (Eds.): Conference on the Use of BCG in Therapy of Cancer. Natl. Cancer Inst. Monogr. No. 39. Wash., D.C., U.S. Govt. Print. Off., 1973, 276 pp.

Opferkuch, W., Snyderman, R., and Borsos, T.: Generation of chemotactic activity by immune complexes carrying clustered or nonclustered $C\overline{42}$ sites. Eur. J. Immunol. 3: 371-372, 1973.

Serial No. NCI-4674
1. Biology Branch, Carcinogenesis, DCCP
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Antigenic Changes in Neoplastic Transformation of Guinea Pig Cells by Chemical Carcinogenesis In Vitro

Previous Serial Number: Same

Principal Investigator: Sarkis H. Ohanian, Ph.D.

Other Investigators: Charles H. Evans, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total	0.9
Professional:	0.6
Other :	0.3

Project Description

Objectives:

Definition of the alteration in surface antigen expression associated with in vitro transformation of guinea pig cells by chemical carcinogens.

Methods Employed:

Antibodies to normal and chemically transformed cells are produced in guinea pigs and rabbits. Specificity of the antisera is obtained by absorption and tested by complement lysis of cells, immunofluorescence and by quantitative immunochemical techniques.

Major Findings:

Two cloned morphologically distinct tumorigenic transformed cell lines originally exposed to carcinogens of different chemical classes and the parental untreated non-tumorigenic nontransformed fetal cell strain are being studied. Cells grow best in 1640 medium with 10% fetal bovine serum. Cells in the log phase of growth are used for immunization and antiserum testing. They are harvested with EDTA followed by washing and resuspension in 1640 without fetal bovine serum. This procedure yields suspensions of cells with greater than 99% trypan blue dye exclusion and less than 1% cytoplasmic staining with fluorescein labelled goat anti-rabbit or rabbit

anti-guinea pig serum. Cell suspensions harvested with trypsin were found to have a higher percentage of dead cells.

Antisera directed against the non-tumorigenic and tumorigenic cells have been produced in rabbits and in syngeneic guinea pigs by a variety of inoculation routes including subcutaneous, intraperitoneal and footpad injections. Neuraminidase-treated cells and cells in complete Freund's adjuvant have also been used for immunization. Antisera produced by these various means have demonstrated about the same high reactivity by employing indirect immunofluorescence (4+) and complement-mediated microcytotoxicity (100%). Antisera absorbed with fresh normal adult syngeneic guinea pig cells and fresh normal syngeneic fetal guinea pig cells exhibit decreased fluorescence to 1+ and cytotoxicity to 0% killing. By C1FT tests no specificity could be detected. Further absorption with the cell line used to produce the antiserum removes activity to all three cell lines. This indicates the presence of cross-reacting or common antigens on the tumorigenic and non-tumorigenic cells not present on normal fetal or adult guinea pig cells. Absorption of some antisera with cells other than the immunizing cell line did not remove all activity to the immunizing cell. However, the activity remaining, to date, is of such low titer (frequently less than 1:2) that it is below the sensitivity of the cytotoxic test and is insufficient to permit identification of specific neoantigens with immunofluorescence.

Significance to Biomedical Research and the Program of the Institute:

It will be possible to answer 1) what specific or general antigenic changes occur with in vitro chemical carcinogenesis and 2) when do the changes occur in relation to the development of transformation and neoplastic growth.

The detection of cell surface antigen changes during the transformation process would be of great importance in the detection of the precancerous state.

Proposed Course of Project:

In attempts to produce antisera of higher titer, additional inoculation procedures will be employed. Several new lines transformed with different classes of carcinogens will also be included.

Serial No. NCI-4676
1. Biology Branch, Carcinogenesis, DCCP
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Detection of Complement Components on Nucleated Cell Surfaces

Previous Serial Number: Same

Principal Investigator: Sarkis H. Ohanian, Ph.D.

Other Investigators: Tibor Borsos, Sc.D. and Berton Zbar, M.D.

Cooperating Units: None

Man Years:

Total	:	0.3
Professional	:	0.3
Other	:	0.0

Project Description

Objectives:

1. To determine whether tumor cells of human and guinea pig origin have detectable complement components on their cell surfaces.

2. To develop techniques for the detection of other complement components bound to the cell surfaces.

Methods Employed:

A new method has been developed to detect human and guinea pig C4 and C3 bound to cell surfaces. Immunochemical methods including DEAE chromatography, electrophoresis and immunodiffusion are employed to isolate antibodies, protein and complement components. Quantitation of antibody and complement components are carried out using radioisotopic labelling quantitative precipitin procedures, C1FT tests, and new immunochemical tests designed to measure C4 and C3.

Major Findings:

Hu and GP C4 and C3 can be bound to the surface of sheep RBC and remain bound for up to two weeks. These cells are employed in an inhibition of lysis test which can detect as little as 6×10^{11} Hu and GP C4 molecules and 10^{11} Hu and GP C3 molecules.

Two DENA induced guinea pig hepatoma cells, L1 and L10, harvested from the peritoneum of guinea pigs have detectable GP C4 and C3 bound to their surfaces. No Hu C4 or C3 could be detected on both cell lines. L10 cells harvested on different days after implantation were examined for bound C4, C3 and host immunoglobulin. Increasing amounts of both C4 and C3 were detected on cells collected on days 3, 6, 10 and 13 after implantation. On day 15 after IP injection there appeared to be a lesser amount of C4 and C3. The immunoglobulin level, as determined by indirect C1FT tests remained the same up to day 13 when two times as much was detected. On day 15 the immunoglobulin levels decreased slightly from that observed on day 13. Preliminary evidence indicates the class of immunoglobulin may be of the IgG class. L10 cells collected 10 days after implantation and incubated at $4^{o}C$ and $37^{o}C$ in culture medium were assayed 1 and 2 days later for persistence of the guinea pig complement components and immunoglobulin. Both C4 and C3 as well as immunoglobulin were lost from the surface of the tumor cells. Loss of cell-bound components occurred at $4^{o}C$ and $37^{o}C$, with the greatest loss occurring with cells incubated at $37^{o}C$. Cell viability dropped about 30% at $37^{o}C$ and about 10% at $4^{o}C$ on the second day.

L10 tumor cells sensitized with rabbit anti-Forssman antibody were assayed for the persistence of the immunoglobulin after incubation at $37^{o}C$ and $4^{o}C$. Using both indirect and direct C1FT, no loss of C1 fixing activity or loss of cell-bound antibody could be detected on day 2, the last day tested.

Significance to Biomedical Research and the Program of the Institute:

Techniques for detecting bound complement components will be very useful in determining whether a humoral immune response has been produced to tumor cells in vivo. Methods used for the detection of humoral immunity to tumor cells do not have the sensitivity of this test procedure and therefore may yield misleading results.

The knowledge obtained from these studies will be helpful in determining the role of humoral immunity in the control of tumor growth.

Proposed Course of Project:

Normal tissue and primary explants of a variety of human and selected guinea pig tumors will be examined for cell-bound C4. New techniques will be developed for detection of late-acting complement components. Additional studies concerning the fate of cell-bound immunoglobulin and complement components will be carried out.

Publications

Ohanian, S. H. and Borsos, T.: Immunochemical quantitation of cell-bound C4. J. Immunol. 112: 979-986, 1974.

Serial No. NCI-4662
1. Biology Branch, Carcinogenesis, DCCP
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Immune Cytolysis

Previous Serial Number: Same

Principal Investigator: Sarkis H. Ohanian, Ph.D.

Other Investigators: Tibor Borsos, Sc.D. and Berton Zbar, M.D.

Cooperating Units: None

Man Years:

Total	:	0.6
Professional	:	0.6
Other	:	0.0

Project Description

Objectives:

A study of the molecular aspects of antibody-complement lysis and antigen topography of nucleated cells.

Methods Employed:

Antibodies to normal and tumor antigens are detected and quantitated by immune cytolysis, colony inhibition, C1 fixation and transfer (C1FT) and by uptake of I^{125} labelled antibody.

Immunochemical methods including Sephadex and DEAE chromatography, electrophoresis, immunodiffusion and ultracentrifugation are employed to isolate antibody protein and complement components.

Major Findings:

The malignant guinea pig cells, L1, L10 and L_2C appear to express less Forssman antigen than their normal tissue counterparts. Both L1 and L10 appear to have the same amount of Forssman antigen. L10 sensitized with anti-Forssman antibody is resistant to lysis by complement while L1 and L_2C are not. By C1FT tests both L1 and L10 sensitized with anti-Forssman antibody have the same number of C1 fixing sites per cell. L10 cells are lysable, however, when sensitized with specific anti-L10 antibody.

L10 cells sensitized with anti-Forssman or specific rabbit anti-L10 antibody are killed by HuC but not guinea pig C. At dilutions of antibodies generating approximately 10^5 C1 fixing sites per cell, the number of GP and Hu C4 and C3 molecules per cell were determined. Large numbers of these C components (approximately 10^6 C4 and 3 x 10^5 to 10^6 GPC3 and HuC3 respectively) were bound to the cells.

L1 cells sensitized with anti-Forssman antibody are more sensitive to lysis by HuC and GPC than L10. However, as with L10 cells, HuC gives a higher degree of lysis than GPC. At dilutions of anti-Forssman antibody generating 5 x 10^4 C1 fixing sites per cell, approximately 60% and 95% of the cells were killed by GPC and HuC respectively. At dilutions generating 1000 C1 fixing sites per cell, GPC did not kill the cells, whereas HuC killed approximately 56%. L1 cells sensitized with specific anti-L1 antibody were only killed by HuC. Determination of the cell-bound GP and Hu C4 and C3 showed striking differences. Anti-L1 sensitized cells had no detectable GP C4 and C3 while 6 x 10^5 and 3 x 10^5 Hu C4 and C3 molecules respectively could be detected. Cells sensitized with high concentrations of anti-Forssman antibody generated equivalent amounts of cell-bound Hu and GP C4 and C3 molecules while at low dilutions Hu C4 and C3 were approximately two times higher than GP C4 and C3.

Treatment of L10 cells with neuraminidase for varying lengths of time resulted in increased lysis of Forssman and anti-L10 antibody sensitized cells with HuC. Increase in expression of anti-Forssman sites was variable in different experiments as determined by C1FT and quantitative absorption tests. Neuraminidase treated cells, while showing increased susceptibility to lysis by anti-Forssman and anti-L10 C lysis were not susceptible to a putative strain-2 guinea pig anti-L10 preparation. Increased sensitivity to lysis following sensitization with rabbit anti-L1 antisera was also noted. Approximately one-half of the total neuraminidase susceptible sialic acid was removed before an increase in cytotoxic activity is detected.

L1 and L10 cells in tissue culture were examined at different times of their growth cycle for sensitivity to killing by anti-Forssman antibody and HuC. No differences in sensitivity with either cell were noted when samples were taken from the lag, early log, late log and early stationary phases of growth. L10 tissue culture cells were more susceptible to lysis than in vivo grown cells.

Significance to Biomedical Research and the Program of the Institute:

The elucidation at the molecular level, of the effects of antibody and complement on both normal and malignant cells may be important in understanding control of cell growth by immunologic mechanisms.

Control of tumor cell growth in vitro makes it possible to study the action of specific and nonspecific agents on the cells at different stages of their growth cycle. The knowledge obtained from in vitro experiments will be very helpful when applied to the study of more complicated in vivo conditions.

Proposed Course of Project:

More extensive work will be carried out on the effect of antibody and complement on lysis of nucleated cells. This will include class of antibody (both Forssman and antitumor) and binding of complement components.

Neuraminidase treatment of L1, L10 and L_2C will be carried out and its effect on antigen expression and sensitivity to C lysis in more detail.

Work will continue on the role of cell growth phase on antigen expression and susceptibility to complement lysis.

Publications

Ohanian, S. H., Borsos, T., and Rapp, H. J.: Lysis of tumor cells by antibody and complement. I. Lack of correlation between antigen content and lytic susceptibility. J. Natl. Cancer Inst. 50: 1313-1320, 1973.

Serial No. NCI-4677
1. Biology Branch, Carcinogenesis, DCCP
2. Tumor Antigen Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mononuclear Cell Chemotaxis

Previous Serial Number: Same

Principal Investigators: Edward J. Leonard, M.D. and David A. Boetcher, M.D.

Other Investigators: Monte S. Meltzer, M.D.

Cooperating Units: None

Man Years:

Total	:	1.0
Professional	:	1.0
Other	:	0.0

Project Description

Objectives:

To develop quantitative measures of reactions occurring in the effector limb of the immune response and to determine whether they are altered in tumor-bearing animals or patients. To study possible mechanisms of altered responsiveness in tumor-bearing animals and patients. To adapt the assay for use with mouse peritoneal mononuclear cells in order to study "activated" macrophages and compare chemotactic responsiveness with killing of tumor cells in vitro.

Methods Employed:

Complement-derived (C5a) and lymphocyte-derived (LDCF) chemotactic factors were generated, filtered and stored in aliquots so that samples of standard preparations would be available over a long period. Venous blood samples were obtained from cancer patients, hospitalized patients without cancer, and normal controls. Peripheral blood mononuclear cells were isolated on Ficoll-Hypaque gradients. Responses to chemotactic factors were measured in modified Boyden chambers, the cell suspensions being separated from the solution of chemotactic factor by a nucleopore membrane with 5 micron pores. Serum samples from cancer patients or controls were heated at 56°C for 30 minutes and then incubated at 37°C with normal indicator cells to test for serum inhibitors of chemotaxis. Mouse peritoneal cells were obtained by peritoneal washout with medium containing 20% fetal calf serum.

Major Findings:

1. The monocyte chemotactic response (MCR) to a standard concentration of LDCF was impaired in 24 of 44 cancer patients studied, compared with 2 of 24 hospitalized patients without cancer and 3 of 22 normal individuals. Impaired responses were noted in patients with a variety of different malignant tumors, with or without metastases. Cancer patients with normal monocyte chemotaxis frequently had localized disease (Stage 0-I).

2. Other findings in this study included the fact that 7 of the 20 cancer patients with normal chemotaxis had localized purulent infection, which suggests that infection may abrogate an impaired chemotactic response. Previous granulomatous disease was also associated with normal MCR's in the cancer patient group.

3. Human basophil leukocytes were also found to respond to C5a and LDCF. Incubation of basophils with LDCF enhances their ability to migrate subsequently to C5a. The addition of sera from some cancer patients to suspensions of leukocytes inhibits chemotaxis. There appears to be an inverse relation between serum inhibitory activity and DNCB reactivity in the patients tested.

4. Mouse macrophages were shown to respond chemotactically to LDCF and endotoxin-activated mouse serum. The response of activated macrophages to LDCF is 3 to 10 times greater than that of normal macrophages.

Significance to Biomedical Research and the Program of the Institute:

The MCR test is a useful measure of one function of the effector arm of the immune response. A defect of the effector system has been demonstrated in many cancer patients which may occur early in the progress of malignancy, affording opportunities for tumor detection, and may relate to patient prognosis. Clarifying the role of the effector arm of the immune response is essential for understanding the function of the immune system in tumor rejection. Studies on basophil chemotaxis may contribute to understanding the role of basophils in cellular immune reactions.

Proposed Course of Project:

Study of mechanisms of decreased MCR in cancer patients. Longitudinal study of abnormal chemotaxis in cancer patients. Further study of mechanisms of basophil chemotactic response. Studies on the role of basophils in immune reactions to tumor-specific transplantation antigens. Study of relationships between basophil chemotactic responsiveness and DNCB skin testing. Use of mouse model to further study the importance of chemotactic responsiveness in macrophage tumor killing.

Publications

Boetcher, D. A. and Leonard, E. J.: Abnormal monocyte chemotactic response in cancer patients. J. Natl. Cancer Inst. 52: 1091-1099, 1974.

Boetcher, D. A. and Leonard, E. J.: Basophil chemotaxis: Augmentation by a factor from stimulated lymphocyte cultures. Immunol. Comm. 2: 421-429, 1973.

Serial No. NCI-4675
1. Biology Branch, Carcinogenesis, DCCP
2. Tumor Antigen Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Immunological Mechanisms of Tumor Rejection

Previous Serial Number: Same

Principal Investigators: Edward J. Leonard, M.D. and Monte S. Meltzer, M.D.

Other Investigators: Howard G. Smith, M.D., Bruce S. Zwilling, Ph.D., Glenn E. Trivers, Robert C. Bast, Jr., M.D. Berton Zbar, M.D., Tibor Borsos, Sc.D., and Robert W. Tucker, M.D.

Cooperating Units: Laboratory of Biology, NCI

Man Years:

Total	: 2.5
Professional	: 2.5
Other	: 0.0

Project Description

Objectives:

To determine what immunological mechanisms occur in tumor cell killing in order to provide a rational basis for elimination of tumor cells by immunological means.

Methods Employed:

Chemically induced murine and guinea pig tumors, maintained in tissue culture or by passage in syngeneic hosts, were used. Antigen recognition responses were monitored by antigen induced lymphoblastic transformation, inhibition of macrophage migration and delayed cutaneous hypersensitivity reactions. Cytotoxic responses were measured by inhibition of cellular uptake of radioactive amino acids, by release of radioactive label from damaged cells, by plaque formation or time-lapse cinematography in tissue culture monolayers.

Major Findings:

1. Xenogeneic antibody against line-10 tumor-specific antigen. In collaboration with the Immunochemistry Section, we produced rabbit antibody against line-10 cells; the antisera were rendered specific for line-10 tumor-specific

antigen by appropriate absorptions. In contrast to the guinea pig antibody noted above, the rabbit antibody mediated a variety of biological effects. Rabbit antibody coated line-10 cells fixed not only guinea pig C1 but also whole complement. Death of the antibody coated cells measured by trypan blue uptake, occurred on the addition of human or rabbit complement and also with guinea pig complement after longer incubation times. Furthermore, spleen cells from unimmunized guinea pigs killed rabbit antibody-coated line-10 cells in the absence of added complement. Thus our in vitro tests indicated two mechanisms by which rabbit antibody could damage line-10 tumor cells: by activation of the complement system or by activation of non-immune lymphocytes. We have now begun studies of the xenogeneic antibody in vivo and have shown that intradermally inoculated line-10 cells mixed with rabbit antibody grew poorly or not at all. It is of further interest that mixtures of tumor cells with specific guinea pig antibody (for which we could demonstrate no biological activity in vitro) grew as well as control cells.

2. Effect of peritoneal exudate (PE) cells from line-10 immune guinea pigs on line-10 cells in vitro. Previous studies from our laboratory have shown the requirement for the development of specific cellular immunity in the rejection of a line-10 tumor cell challenge. Despite considerable effort during the past year, we were unable to demonstrate consistently the in vitro counterpart of this phenomenon by measuring cytotoxic effects of PE cells from line-10 immune animals on suspensions of line-10 cells. Low level, statistically significant cytotoxicity was seen with some preparations, but the magnitude and frequency of positive results was too low for this to be a practical experimental model.

3. Effect of PE cells from BCG immune guinea pigs on line-10 cells in vitro when activated by PPD. A part of this study was done in collaboration with the Cellular Immunity Section, and was directed toward the immunologically non-specific destruction of target cells that may occur at the site of a cellular immune reaction in which the stimulating antigen is unrelated to the target cell. Our system comprised PE cells from BCG immune guinea pigs which were stimulated by the addition of PPD. When line-10 tumor cells were added to this mixture, a significant proportion of the tumor cells were destroyed during the ensuing 48 hours. To determine the cell type causing the cytotoxicity, the PE cells were separated into a non-adherent or lymphocyte-rich fraction and an adherent fraction comprised almost entirely of macrophages. The cytotoxic action of the PPD stimulated BCG immune PE cells was accounted for by the non-adherent population and no cytotoxic role could be assigned to the adherent macrophages. This result should be contrasted to histological studies of line-10 tumor cells being destroyed subsequent to the inoculation of BCG which suggests that histiocytes may play a role in the destruction of the tumor cells; and to the role of activated macrophages in other in vitro systems as noted, for example, below. Tumor, transformed and normal syngeneic target cells, were assayed to determine their relative susceptibility to lymphocyte cytotoxicity. In general, tumor cells were most susceptible, morphologically transformed cells intermediate and normal cells least susceptible to this immune reaction.

4. Macrophage-tumor cell interactions in vitro were analyzed by a plaque assay or by time-lapse cinematography. Plaque formation occurs when peritoneal macrophages from BCG immune mice boosted intraperitoneally with BCG are mixed with tumor cell suspensions or added to confluent tumor cell monolayers. IP injection of endotoxin or poly I into normal mice also activate macrophages. An equal number of macrophages from normal mice or from oil, starch and thioglycollate-induced peritoneal exudates have no effect. Tumor monolayer plaques are the sequella of cytostatic and cytotoxic action. These effects require close macrophage-tumor cell contact and become apparent after 24-36 hours of culture. Soluble supernatant factors have not been demonstrated. Target cell susceptibility to BCG-activated macrophages correlates closely with in vivo tumorigenicity. Syngeneic, allogeneic and xenogeneic normal cell lines are quantitatively less susceptible to activated macrophages than syngeneic, allogeneic or xenogeneic tumor cell lines.

5. Tumor suppression mediated by BCG is more effective in vitamin A treated mice than in controls. Vitamin A may act by augmenting the animal's immune responses to BCG and/or increasing the susceptibility of the tumor to specific and nonspecific immune attack.

Significance to Biomedical Research and the Program of the Institute:

Analysis of the effector immune events leading to tumor rejection may provide a rational basis for manipulation of host responses or tumor cell in an effort to eliminate or prevent progression of the tumor.

Proposed Course of Project:

Physicochemical characterization of soluble cytotoxins, determination of their mechanism of action, administration of cytotoxins in animal tumor models. Analysis of the cytotoxic action of PE cells, stimulated either by tumor antigens or by antigens unrelated to the tumor. Further characterization of antibody-mediated lymphocyte cytotoxicity to ascertain its role in vivo.

Publications

Cleveland, R. P., Meltzer, M. S., and Zbar, B.: Tumor cytotoxicity in vitro by macrophages from mice infected with Mycobacterium bovis, strain BCG. J. Natl. Cancer Inst., (In Press).

Littman, B. H., Meltzer, M. S., Cleveland, R. P., Zbar, B., and Rapp, H. J.: Tumor-specific cell-mediated immunity in guinea pigs with tumors. J. Natl. Cancer Inst. 51: 1627-1635, 1973.

Paque, R. E., Meltzer, M. S., Zbar, B., Rapp, H. J., and Dray, S.: Transfer of tumor-specific delayed hypersensitivity in vitro to normal guinea pig peritoneal exudate cells using RNA extracts from sensitized lymphoid tissues. Cancer Res. 33: 3165-3171, 1973.

Serial No. NCI-4667
1. Biology Branch, Carcinogenesis, DCCP
2. Tumor Antigen Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Isolation and Study of Tumor-Specific Antigens

Previous Serial Number: Same

Principal Investigators: Edward J. Leonard, M.D. and Tibor Borsos, Sc.D.

Other Investigators: Arleen K. Richardson, Ph.D., Monte S. Meltzer, M.D. Howard G. Smith, M.D., and Barbara D. Hooks, Ph.D.

Cooperating Units: None

Man Years:

Total	:	3.0
Professional	:	2.0
Other	:	1.0

Project Description

Objectives:

To obtain tumor-specific antigens in soluble and partially purified form. To use these antigens in inducing, establishing and measuring tumor-specific immunity in guinea pigs. Knowledge derived from the guinea pig model will be applied to the isolation, purification and characterization of human tumor-specific antigens. These antigens will then be used in diagnostic studies of human tumor epidemiology, in monitoring patient response to treatment and as an aid in treatment. Initial studies will be on the ascites cell form of diethylnitrosamine induced hepatoma of strain-2 guinea pigs.

Methods Employed:

Initial extraction: 3M KCl and other hypertonic salts. Further purification: ammonium sulfate precipitation, gel filtration, DEAE chromatography. Assay methods: skin testing; in vitro correlates of cellular immune responses: lymphocyte blastogenesis, inhibition of macrophage migration; immunization of guinea pigs with extracts and challenge of test animals with live tumor cells. Production and testing of heterologous antisera to tumor antigen: immunization of rabbits with whole tumor cells, absorption of sera with various cell suspensions, testing of absorbed sera by C1 fixation and transfer test and by immune fluorescence.

Major Findings:

Tumor-specific antigen(s) extracted from guinea pig line-10 tumor with 3M KCl is heterogeneous with respect to size as determined by gel filtration, density as determined by sucrose density ultracentrifugation and precipitability by ammonium sulfate. The heterogeneity suggests that either the antigen is fragmented or remains attached to solubilized membrane components of varying size. The yield of antigen, assayed by skin test in a line-10 immune guinea pig, is 10-20%; and since the missing antigen cannot be accounted for in the extracted cell pellet, it is probably destroyed during the extraction procedure.

A new source of soluble line-10 tumor antigen was uncovered in the ascites fluid of guinea pigs with tumor ascites and in the culture fluid of cultured line-10 tumor cells. The fluid after a 10-fold concentration yields a precipitate with rabbit anti-line-10 antibody and the precipitate can be seen in agar gel. Absorption of the serum with guinea pig serum and line-1 cells did not alter the precipitate with line-10 cell fluid concentrate. The concentrate was filtered through Sephadex G-200 and antigen was in the excluded portion of the eluate. Evidence was also obtained for the presence of more than one tumor-specific antigen for in two dimensional gel diffusion precipitin analyses two lines of precipitate were found. The antigen is now being further purified and its biological significance in the tumor-bearing animal is being studied.

Guinea pig serum antibody against tumor-specific antigens on the surface of line-10 tumor cells was demonstrated with an indirect immunofluorescence assay. Antibody was found in animals immunized with a line-10 BCG vaccine and in animals progressively growing and metastasizing tumors. Vaccinated animals developed higher antibody levels after multiple inoculations of tumor cells alone. Immunization of line-10 antigen extracts in complete Freund's adjuvant produced delayed cutaneous hypersensitivity responses but no humoral antibody until after challenge with viable tumor cells.

Antibody on the surface of line-10 cells fixed activated C1 but did not fix whole guinea pig complement. Line-10 cells sensitized with the antibody showed no cytotoxic or cytolytic changes in the presence of guinea pig, rabbit or human complement.

The antibody mediated movement of line-10 antigen within the tumor cell membrane in a manner similar to the rabbit anti-line-10 antibody. The movement occurred only after application of a fluoroscein-conjugated antiglobulin reagent and was not mediated by the antibody alone.

Comparison of antigen assays: we have assayed line-10 KCl extracts by skin test and also by antibody absorption assays using rabbit anti-line-10 and guinea pig anti-line-10 antibodies. The antibody absorption curves obtained with increasing amounts of extract are compared with those for intact cells and yields of antigen in the extracts are expressed in cell equivalents. Guinea pig antibody assay and skin test give comparable yields. The yields from the rabbit antibody absorption data are 3- to 10-fold higher.

A new method for obtaining line-10 cell membranes free of nuclei and the bulk of other intracellular constituents was also devised. The method involves slow addition of sucrose to the cell suspension until the concentration is 17.5%, after which the cells are centrifuged and the cells in the pellet are ruptured osmotically by the addition of water. After a period of equilibratio to allow release of intracellular granules, $MgCl_2$ is added to prevent swelling and rupture of the nuclei and the suspension is then subjected to a mild shearing force which results in release of nuclei from the surrounding cell membrane. Nuclei and membranes can then be separated by a one minute centrifugation without the usual complication of sucrose density gradients. Unfortunately this method is not applicable to all cells; the membranes of line-1 tumor, for example, are broken into multiple fragments at the osmotic shocking stage and this makes separation of membranes from intracellular organelles impossible with our simple techniques. But with line-10 cells the method provides virtually intact membranes or large fragments in yields of 15-25%. Furthermore, if the membranes are counted (by phase contrast in a platelet counting chamber) the antigenic activity by skin test or antibody absorption tests is comparable to that of intact cells.

The capacity of KCl extracts and membranes to protect guinea pigs against tumor cell challenge was studied. In several experimental series with line-10 KCl extracts, it was found that immunization with line-10 extract emulsified with CFA or mixed with Ribi vaccine led to delayed cutaneous hypersensitivity when animals were challenged with viable cells and tumor growth did not occur or was delayed in a significant proportion of the animals. All of the animals were not protected, however, in contrast to 100% protection after immunization with viable cells mixed with Ribi vaccine. Therefore, we compared immunogenicity of whole cells, KCl extract and cell membranes mixed with Ribi vaccine, in the expectation that the cell membranes would do better than the KCl extract, since membrane antigenic activity appeared to be intact. To our surprise, the membranes immunized poorly and did not protect at all against tumor challenge. This interesting finding poses the question whether the cell membrane tumor-specific antigen was altered in subtle ways not detected by skin test or antibody absorption or whether immune processing of the cell membrane is different from that of the viable cells.

Significance to Biomedical Research and the Program of the Institute:

Comparison of the newly developed assays for tumor antigen may provide new knowledge about the nature and number of tumor-specific antigens on a chemically induced tumor. The availability of tumor cell membranes retaining tumor-specific antigenic activity provides a valuable preparation for the study of immunogenicity.

Proposed Course of Project:

Further work on the comparison of assays to determine whether there is more than one tumor-specific antigen on line-10 cells. Studies on the immunogenicity of line-10 membranes.

Publications

Borsos, T., Richardson, A. K., Ohanian, S. H., and Leonard, E. J.: Immunochemical detection of tumor-specific and embryonic antigens of diethylnitrosamine-induced guinea pig tumors. J. Natl. Cancer Inst. 51: 1955-1960, 1973.

Kripke, M. L. and Borsos, T.: Guest editorial: Immune surveillance revisited. J. Natl. Cancer Inst., (In Press).

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Leonard, E. J.: Cell surface antigen movement: Induction in hepatoma cells by antitumor antibody. J. Immunol. 110: 1167-1169, 1973.

Serial No. NCI-4668
1. Biology Branch, Carcinogenesis, DCCP
2. Tumor Antigen Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Calcium Transport Globulin System (Cardioglobulin)

Previous Serial Number: Same

Principal Investigators: Edward J. Leonard, M.D. and Stephen Hajdu, M.D.

Other Investigators: None

Cooperating Units: Laboratory of Kidney and Electrolyte Metabolism, NHLI

Man Years:

Total	:	0.2
Professional	:	0.2
Other	:	0.0

Project Description

Objectives:

To develop immunochemical assay methods for free and cell-bound calcium transport globulin system (CTGS); to apply these methods to the isolation and purification of CTGS components; and to look for CTGS alterations in disease.

Methods Employed:

Isolated frog hearts were used for bioassay for human CTGS components. In addition to standard techniques, new methods of protein fractionation were devised to separate CTGS components from other serum proteins.

Major Findings:

The interaction of the CTG components with the assay frog heart can now be summarized as follows:

1. B1 + B2 + frog heart $\longrightarrow$ B-heart

   (1a. human serum + frog heart $\longrightarrow$ B-heart + I_A)

2. B-heart + C-Ca + A$\sim$P $\longrightarrow$ B-heart C-Ca + A + P_i

The new findings in this schema are: We can now produce the reaction with four purified protein components, each of which can be functionally and chemically freed of the other three. Reaction 1 requires two proteins; B2 is a new addition to the family. When whole human serum rather than B1 + B2 is added to the frog heart (reaction 1a), an inhibitor of component A is elaborated which prevents reaction 2 from going to completion.

Component purification. Human serum was divided into two groups of proteins by precipitation at a sodium sulfate concentration of 15%. The supernatant proteins were used without further purification after removal of the sodium sulfate. The precipitated proteins were fractionated into euglobulins and pseudoglobulins. It was found that reaction 1 required the addition of the euglobulin fraction and the 15% supernatant proteins; both fractions were also required for reaction 2. For both reactions the fractions had to be present together in the reaction mixture. The chemical similarity of the euglobulin components made it difficult to answer whether different euglobulin proteins were required in reaction steps 1 and 2. They were both excluded by G-200 gels and both were pelleted along with other high molecular weight proteins on ultracentrifugation. Their lability ruled out many subfractionation procedures. The problem was solved by taking advantage of the fact that reaction 1 was prevented by heparin. Studies with dextran sulfate, a macromolecule with properties similar to heparin, showed that the euglobulin required for reaction 1 (now called B1) could be precipitated by dextran sulfate and then recovered with full biological activity after protamine sulfate was added to dissolve the precipitate. The euglobulin required for reaction 2 (component C) was not precipitated by dextran sulfate. This procedure established that B1 and C are chemically distinct entities.

We also showed during this year that reactions 1 and 2 require chemically different components from the albumin-rich 15% sodium sulfate supernatant. Component A, the high energy phosphoprotein that participates in reaction 2, is inactivated by dextran sulfate and can be removed from solution by a sulfonated G-50 Sephadex (cross-linked dextran) prepared for us by Pharmacia. Component B2, required for reaction 1, is unaffected by dextran sulfate. The two proteins also have different elution patterns from DEAE (B2 binds more firmly). The inhibitor produced during reaction 1a affects component A but not B2.

Antibody production for tissue localization. We have completed preliminary studies with rabbit antibody to human B1. The B1 was purified by G-200 chromatography, dextran sulfate precipitation, solubilization with protamine and salting out with sodium sulfate. The resulting antiserum inhibited the biological activity of B1 and thus has anti-B1 antibody. It localized on cell surfaces of human heart and skeletal muscle. However, the antiserum makes three precipitin lines with electrophoresed human serum and is therefore not yet sufficiently pure for establishing the tissue localization of the CTG system.

Significance to Biomedical Research and the Program of the Institute:

Although there is much speculation in scientific literature about the possible role of calcium as a regulator of cell functions, including secretory activity, motility and contact inhibition, there is practically no solid evidence. We have shown that CTGS is a serum protein system that can affect transmembrane calcium movement in isolated frog hearts. Isolation and characterization of the components of this system and the development of antisera to the components should enable us to answer questions about its tissue localization and biological function.

Proposed Course of Project:

Further purification of CTGS components, production of monospecific antisera to these components; use of the antisera to determine tissue localization of CTGS and to measure free- and tissue-bound CTG components in human disease.

Serial No. NCI-4629
1. Biology Branch, Carcinogenesis, DCCP
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Mechanism of Cell Transformation

Previous Serial Number: Same

Principal Investigator: Joseph A. DiPaolo, Ph.D.

Other Investigators: Paul J. Donovan, B.S., Charles H. Evans, M.D., Ph.D. and Nicolae C. Popescu, Ph.D.

Cooperating Units: None

Man Years:

Total:	4.0
Professional:	2.5
Other:	1.5

Project Description

Objectives:

The primary objective of this project is to establish conditions and methods for in vitro quantitative study of chemical transformation. The immediate targets of study are:

1. Development of rapid assay systems for chemical carcinogens suitable for the screening of compounds and populations.

2. Define the required conditions for the transformation of human fetal cells.

3. Determination of ways to increase the susceptibility of primary cell lines or cell strains to chemical transformation.

4. Development of assays to determine whether in vitro transformation is accompanied by the appearance of tumor specific antigens.

5. Determination of the role of non-oncogenic and oncogenic viruses in chemical transformation.

6. Determination of the ultrastructural changes of cells during the course of in vitro chemical transformation so that they may be contrasted with normal cells and with information pertaining to viral transformation.

7. Determination of the role of radiation and alkylating compounds in enhancing neoplastic transformation by chemicals.

8. The transformation of epithelial-like cells which result in the formation of carcinomas when the cells are transplanted into animals.

9. Analysis of variations in populations of somatic cells and their correlation with genetic changes.

Methods Employed:

All procedures performed are with the view of quantitative phenomena in vitro. Such procedures are required in order to determine whether or not the transformation observed is due to the direct or indirect effect of the carcinogen and in order to study the early events associated with in vitro transformation. Cells used come from freshly isolated cells from animals and humans that as controls have many of the attributes of "normal" cells and from cell lines which are known to exhibit some of the properties associated with non-transformed cells. Discrete cells are grown in complete medium in the presence or absence of irradiated rat or hamster cells. The cells may be derived from whole embryos or may be from specific organs. The cells are exposed to chemical carcinogen transplacentally or prior to or subsequent to seeding the cells in petri dishes.

Approximately one week subsequent to treatment the cells are examined under phase or under stained conditions for number of transformed colonies, toxicity, and spectrum of morphology of both the normal and transformed colonies. The frequency of transformation is expressed in a number of different ways. These take into consideration the observed rate of transformation on a per-cell basis or on a number of colonies obtained.

Major Findings:

Previous data has demonstrated that the pretreatment of cells by X-irradiation results in an increase in the number of transformations by benzo(a)pyrene. We now report that the absolute increase in the number of transformed colonies is evidence that irradiation does not cause selection of irradiation-resistant cells more likely to be transformed by chemical carcinogen. The enhancement obtained with benzo(a)pyrene is independent of the concentration of benzo(a)pyrene and the enhancement data is consistent with the one hit hypothesis. Because methyl methanesulfonate and ethyl methanesulfonate, are known to be relatively weak carcinogens, the influence of these two alkylating agents on transformation of benzo(a)pyrene and a number of other known potent carcinogens was determined. These alkylating agents were selected because they induce physiochemical effects similar to those produced by ionizing radiation in cell DNA. A one hour pretreatment with either compound will enhance the transformation of a number of potent carcinogens. It is immaterial whether hamster cells are pretreated in mass culture with an alkylating agent or cells that have been plated for colony formation are treated; in both cases the maximum enhancement occurs when the potent

carcinogen is added 48 hours after the treatment with the alkylating agent. The results obtained with MMS or EMS differ from those with pretreatment with X-irradiation in that a higher enhancement ratio is obtained with X-irradiation and enhancement is demonstrable if a second carcinogen is applied 6, 12, or 24 hours after X-ray treatment, whereas with MMS, no enhancement is obtained at these earlier times. The enhancement produced by pretreatment with an alkylating agent is slightly less than that produced by X-irradiation pretreatment. The exact cause of the enhancement in transformation resulting from the interaction of these agents is not yet known. Direct evidence for early repair of breaks of cell DNA following MMS treatment was obtained by sedimentation of DNA from MMS treated cells in alkaline sucrose gradients. There is a marked decrease in the molecular weight of the cell DNA when tested immediately following treatment; however, within 6 hours the sedimentation profile returns to nearly controlled values and by 48 hours after MMS treatment no alkaline label regions could be demonstrated. It can be concluded that repair of DNA damage as it is now understood is probably not involved. The possibility that the enhancement observed at 48 hours is due to some artifact is remote. Preliminary experiments with ultraviolet indicate no such enhancement. Other experiments in which caffeine is added to cultures subsequent to the treatment with a carcinogen indicate that although enhancement occurs it is at a different time interval than that which was obtained with irradiation or with alkaline agents.

The characterization of chemically transformed cells has been pursued from a number of different directions. Recently, we reported that chemically transformed Balb/3T3 cells that multiply well in medium supplemented with 10% agamma serum produced a higher efficiency in the uptake of 2-deoxy-D-glucose than the controls, chemically transformed cells show only a 50-100% difference even when the cultures are growing exponentially. This characteristic is maintained in crowded cultures; untransformed Balb/3T3 cells cease taking up the sugar, while chemically transformed cells continued at the same rate even at high cell densities. Reduction of the serum concentration in the medium from 10-2% has only a slight effect on the uptake of the deoxyglucose by chemically transformed cells. Chemical carcinogens appeared to alter permanently the uptake mechanism for a key nutrient but not to the same extent as virally transformed cells.

Transformation induced by carcinogenic chemicals has been extended to rat fetal cells in tissue culture. At the same time the same carcinogen, DMBA, was injected subcutaneously into male weanling rats. After transformation was obtained in vitro, it was demonstrated that the cells were capable of producing fibrosarcomas in rats that were identical to those which had been produced by the direct injection of the carcinogen into weanling rats. Thus it was possible to compare by means of chromosome banding of cells prior to transformation and after transformation to cells obtained from in vivo tumors. One line transformed in vitro and three tumors induced in vivo were mainly diploid, with a variably proportion of polyploid cells. Two

other in vitro transformed lines and two in vivo induced tumors each showed particular numerical and/or structural chromosomal aberrations preferably involving the longest chromosomes, numbers 1, 2, or 3. The same chromosomes were affected in the non-transformed and non-tumorigenic lines isolated from carcinogen treated dishes. The untreated control lines showed no specific changes except for an accumulation of polyploid cells. Since neither specific alteration of the chromosome distribution, nor a particular and constant chromosomal aberration was found, it is concluded that in vivo or in vitro malignant transformation by DMBA leading to the formation of fibrosarcomas is not directly caused by gross abnormalities of the chromosomes. This conclusion is consistent with our previous observations on hamster transformed cells. Although the presence of some minor chromosomal rearrangements cannot be excluded even by the use of the refined banding techniques, it is highly improbable that neoplastic transformation is directly caused by specific gross abnormalities of the chromosomes. Studies of chromosomes of Syrian hamster cells using techniques for constitutive heterochromatin have permitted further analysis of both normal and marker chromosomes found following neoplastic transformation and in tumors derived from the transformed cells. The Syrian hamster cells that have been transformed by a variety of chemical carcinogens, had chromosomes with constitutive heterochromatin characteristic of the species. Heterochromatin alterations occurred in only two different tumor cultures and in no transformed line and thus cannot be considered a causative factor in the transformation phenomenon. The C band technique facilitated the analysis of marker chromosomes by providing additional information concerning some of the markers that were first recognized by the horizontal banding analysis.

Variant cell lines obtained by treating transformed Balb/3T3 mouse cells with FUdR have growth parameters closely resembling non-transformed cells. Chromosome analysis of these variant lines demonstrated that some variants may have an increased or a decreased number of chromosomes compared to the parental transformed cell lines. The variants isolated after FUdR treatment maintained certain growth-controlled properties and did not produce tumors suggesting that the alterations, whether or not they are due to mutations are quite stable. All variants have unique marker chromosomes not present in the parental transformed Balb/3T3 cells. The distribution of marker chromosomes and heterochromatin suggested that the initial event in variant formation was a reduction in chromosome number with a subsequent polyploidization of the reduced chromosome complement.

The question of how to evaluate the tumorigenic potential of xenogenic mammalian cells is under study utilizing nude mice. In this study, nude (nu/nu C3H/Swiss mice) were compared with hamsters and guinea pigs in their ability to serve as recipients for evaluating the tumorigenicity of hamster and guinea pig cells transformed in cell cultures by chemical carcinogens and HeLa cells obtained from the Cancer Chemotherapy Service Center. The transformed animal cells were obtained from either in utero exposure to a chemical carcinogen or by direct application of a chemical following introduction of cells into culture. Morphologically transformed cell strains

derived from cultures exposed to diverse carcinogens (benzo(a)pyrene, diethylnitrosoamine, N-methyl-N'-nitro-N-nitrosoguanidine) were subsequently grown in roller culture and tumorigenicity assessed in the parent species and in nude mice. Animals were inoculated subcutaneously with 10^5 and 10^8 transformed cells and observed for the appearance of tumors. Both transformed hamster and guinea pig cells and HeLa cells produced progressively growing tumors in nude mice. Fewer transformed cells were required to obtain tumors in nude mice than in noninbred Golden Syrian hamsters or in inbred strain-2 guinea pigs. Nude mice provide an additional host for evaluating the tumorigenic potential of xenogeneic transformed cells, a useful resource for the study of carcinogenesis in both syngeneic and noninbred systems.

The host mediated in vivo - in vitro combination bioassay for identifying potential carcinogenicity of chemicals is being utilized to test additional carcinogens and noncarcinogens and is being refined by examining specific organs. The latter is being done to select transformation of epithelial-like cells which may result in the formation of carcinomas when the cells are transplanted into animals. Using this last refinement on fetuses late in gestation, it has been possible to obtain transformations from specific organs. Many of the transformations appear epithelial-like. These will be further cloned and recloned prior to injection into animals in order to obtain evidence as to the type of tumor that will be produced. This approach is being utilized since it is well established that differentiated tumors may retain their characteristics in cultures whereas differentiated normal tissues soon after being placed in culture lose many of their normal associated functions.

In the past year, it has been possible to obtain tissue from four different fetuses. These have been treated with polycyclic hydrocarbons, such as benzo(a)pyrene or methylcholanthrene, with MNNG, and acetoxy-AAF. In some instances toxicity as indicated by cell lethality has occurred within three or four days. In other cases, lethality occurred only when the compound is added to the same culture the second week; while in rare cases no toxicity is observed. As a result of the treatment, there is a phenotypic variation in the cell pattern which may or may not be permanent. In those cases in which the variation appears to be of a non-transitory nature, cells have been returned to conditioned hamsters or nude mice. Thus far, no tumors have been produced but in rare cases the treated line continues to grow whereas the control line has a finite lifetime. These results are encouraging from the point of view that they have indicated that carcinogens are capable to extending the life span of cells which of course is one characteristic of non-normal cells. Additional experiments are underway to determine if the conditions that influence hamster transformation can be utilized in combination with carcinogens directly to human cells.

Significance to Biomedical Research and the Program of the Institute:

The need to study chemical carcinogenesis in vitro is obvious when one realized that carcinogenesis is really a problem in cell ecology.

Consequently, in vitro carcinogenesis provides an opportunity to study chemical-target cell interaction and chemical-nontarget cell problems such as other effects of the chemical on cells without the complications of in vivo problems. Therefore, in vitro carcinogenesis has wide applications in terms of studying compounds in man's environment for the sake of control as well as for determining how they alter the physiological process of cells.

Proposed Course of Project:

It is planned to further study the conditions which are responsible for or can alter in vitro transformation with specific emphasis on the requirements of human fetal cells. These will include the use of X-rays, combinations of carcinogens of different classes, the combination of in vivo - in vitro systems, and the effect of stimulators and inhibitors of mixed function oxidases on toxicity and transformation. The mechanisms of early events of carcinogen-cell interaction will be studied. Investigation of different proximate carcinogens and metabolic inhibitors is planned. In addition, experiments are planned to test whether neoplastic transformation has occurred as a result of direct induction by chemicals, as the result of selection or of viral activation. The testing of the tumorigenicity of cells exposed for different time intervals will continue.

Honors and Awards

Invited Lecture, "Quantitative aspects of in vitro chemical carcinogenesis." George Washington University Medical Center, Washington, D. C. 1973.

Invited Lecture, "Model systems in carcinogenesis." Case Western Reserve University, Cleveland, Ohio 1973.

Invited speaker on "carcinogenesis" as part of the scientific program for the 75th Anniversary of Roswell Park, Buffalo, New York 1973.

Invited Speaker, "Studies on the quantitation of in vitro transformation by chemicals." Filene Hall, Skidmore College, Saratoga Springs, New York 1973.

Invited Speaker, "Evaluation of carcinogenic potential of drugs, II. Short-term tests for carcinogenesis: Tests involving induction of neoplasia." National Academy of Sciences, Washington, D. C. 1973.

Invited Speaker, "Mammalian cell models for chemical carcinogenesis." Workshop on Approaches to Assess the Significance of Experimental Chemical Carcinogenesis Data for Man, Brussels, Belgium 1973.

Publications

Casto, B. C., and DiPaolo, J. A.: In vitro transformation: Interaction of chemicals, viruses, and irradiation. In Proceedings of the VIth International Symposium on Comparative Leukemia Research, Nagoya, Japan (In Press).

Casto, B. C., and DiPaolo, J. A.: Virus, chemicals and cancer. Progr. med. Virol. 16: 1-47, 1973.

Casto, B. C., Pieczynski, W. J., and DiPaolo, J. A.: Enhancement of adenovirus transformation by pretreatment of hamster cells with carcinogenic polycyclic hydrocarbons. Cancer Res. 33: 918-834, 1973.

Casto, B. C., Pieczynski, W. J., and DiPaolo, J. A.: Enhancement of adenovirus transformation by treatment of hamster embryo cells with diverse chemical carcinogens. Cancer Res. 34: 72-78, 1974.

DiPaolo, J. A.: Mammalian cell models for chemical carcinogenesis. In Proceedings of the Workshop on Approaches to Assess the Significance of Experimental Chemical Carcinogenesis Data for Man, Brussels, Belgium. Lyon, International Agency for Research on Cancer 1974 (In Press).

DiPaolo, J. A.: Quantitative aspects of in vitro chemical carcinogenesis. In Ts'o, P. O., and DiPaolo, J. A. (Eds.): Chemical Carcinogenesis. New York, Marcel Dekker Inc., 1974, pp. 443-455.

DiPaolo, J. A., Nelson, R. L., Donovan, P. J., and Evans, C. H.: Host-mediated in vivo - in vitro assay for chemical carcinogenesis. Arch. Path. 95: 380-385, 1973.

DiPaolo, J. A., and Popescu, N. C.: Distribution of chromosome constitutive heterochromatin of Syrian hamster cells transformed by chemical carcinogens. Cancer Res. 33: 3259-3264, 1973.

DiPaolo, J. A., Popescu, N. C., and Nelson, R. L.: Chromosomal banding patterns and in vitro transformation of Syrian hamster cells. Cancer Res. 33: 3250-3258, 1973.

Evans, C. H., and DiPaolo, J. A.: Mammalian cells for carcinogen identification. J. Am. Med. Assoc. 225: 631-632, 1973.

Olinici, C. D., and DiPaolo, J. A.: Chromosome banding patterns of rat fibrosarcomas induced by in vitro transformation of embryo cells or in vivo injection of rats by DMBA. J. Natl. Cancer Inst. (In Press).

Olinici, C. D., Evans, C. H., and DiPaolo, J. A.: Chromosome patterns of nontransformed variants from chemically transformed Balb/3T3 cells. J. Cellular Physiol. (In Press).

Oshiro, Y., and DiPaolo, J. A.: Changes in the uptake of 2-deoxy-D-glucose in Balb/3T3 cells chemically transformed in culture. J. Cellular Physiol. (In Press).

Oshiro, Y., and DiPaolo, J. A.: Loss of density-dependent regulation of multiplication of Balb/3T3 cells chemically transformed in vitro. J. Cellular Physiol. 81: 133-138, 1973.

Popescu, N. C., and DiPaolo, J. A.: G and C chromosomal banding produced sequentially in living and fixed human cells. The Lancet (In Press).

Popescu, N. C., and DiPaolo, J. A.: Radioautography of incorporation of 7,12-dimethylbenz(a)anthracene-^{3}H label into Syrian hamster embryo cells during exposure to inhibitors of protein synthesis. J. Natl. Cancer Inst. 50: 1463-1469, 1973.

Yamamoto, N., Anderson, M. D., and DiPaolo, J. A.: Phage and bacterial inactivation and prophage induction by chemical carcinogens. Molecular Pharmacology (In Press).

Serial No. NCI-4673
1. Biology Branch, Carcinogenesis, DCCP
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Neoplastic Transformation of Guinea Pig Cells by Chemical Carcinogens In Vitro

Previous Serial Number: Same

Principal Investigator: Charles H. Evans, M.D., Ph.D.

Other Investigators: Joseph A. DiPaolo, Ph.D.

Cooperating Units: None

Man Years:

Total:	2.1
Professional:	0.6
Other:	1.5

Project Description

Objectives:

The primary objective of this project is to establish conditions and methods for the quantitative study of in vitro chemical transformation of guinea pig cells. The investigation complements the previously developed and current in vitro chemical carcinogenesis system established within the Cytogenetics and Cytology Section employing Syrian hamster embryo cells and the tumor immunology program utilizing in vivo chemical carcinogen induced tumors developed in strain-2 guinea pigs.

The specific objectives of the project are twofold:

1. The development of a rapid assay system for chemical carcinogens suitable for the screening of compounds and populations. As an inbred strain with a low incidence of spontaneous malignancy strain-2 guinea pigs offer a mammalian system in addition to the Syrian hamster for the analysis of chemicals as potential carcinogens and investigation of the mechanisms of chemical carcinogenesis at the cellular level.

2. The development of assays to determine whether in vitro chemical transformation is accompanied by the appearance of tumor specific antigens. The Biology Branch has extensive experience with tumor immunology in strain-2 guinea pigs. A variety of in vivo and in vitro immunological techniques are presently being utilized. The availability of these techniques and the

syngeneic system offered by the inbred strain-2 guinea pig make this system a better choice for immunological study at this time than the Syrian hamster. The development of neoplastic cells transformed in vitro by chemical carcinogens will permit assessment of cell surface antigen alterations associated with chemical carcinogens as well as further understanding of in vivo tumor immunity in strain-2 guinea pigs as a model system for carcinogenesis and tumor immunology in general.

Methods Employed:

The quantitative in vitro chemical carcinogenesis system developed employing Syrian hamster embryo cells within the Cytogenetics and Cytology Section has been utilized throughout this study. Cells are freshly isolated from whole or specific organs of strain-2 guinea pig embryos or fetuses and are grown in the presence or absence of irradiated rat, hamster or strain-2 guinea pig feeder cell layers. The cells as a mass culture or as individual cells are exposed to chemical carcinogen subsequent to seeding the cells in the petri dish.

Approximately one week subsequent to treatment colonies are examined under phase contrast microscopy or light microscopy following fixation and straining of the cells for the number of transformed colonies, toxicity and spectrum of morphology of both the normal and transformed colonies.

Major Findings:

1. The successful mass cell culture of strain-2 guinea pig embryo and fetal cells in Dulbecco-Vogt and minimal essential medium as well as other media incorporating 10% fetal bovine serum.

2. The ability of primary and secondary mass culture cells to clone with a high degree of efficiency in a variety of media with and without feeder layers. Cloning efficiencies of untreated cells as high as 50% with a feeder layer and up to 30% in the absence of a feeder layer have been regularly observed.

3. The development of toxicity following the addition of chemical carcinogens in both mass and cloned cell cultures was similar to that observed in hamster cells exposed to the same chemicals. Carcinogens from several chemical classes have been employed including the polycyclic compounds benzo(a)pyrene, 3-methylcholanthrene and 7,12-dimethylbenz(a)anthracene as well as the nonpolycyclic chemicals such as N-acetoxy-N-2-fluorenylacetamide and N-methyl-N'-nitro-N-nitrosoguanidine. Fetal cells have also been exposed to chemical carcinogen while in utero via the transplacental route following intraperitoneal inoculation of pregnant guinea pigs. The procedure is the same as that developed for the transplacental host mediated in vivo - in vitro hamster bioassay with excision of the guinea pig fetuses 48 hrs subsequent to maternal inoculation and identification of transformation following introduction of the fetal cells into culture.

4. Morphological cellular changes of guinea pig cells exposed to chemical carcinogens and compatible with in vitro chemical neoplastic transformation as observed in Syrian hamster embryo cells have been seen with cells exposed to chemical carcinogen but not to non-carcinogens either while in utero or after introduction into culture. Twenty cell strains have been evaluated during 12-24 months of cultivation. Morphological alterations in carcinogen treated cultures appear shortly after treatment but transformation, a total loss of cell orientation, does not appear for four or more months, is not seen in controls, and does not appear coincident with capacity of transformed cells to grow as tumors in irradiated newborn guinea pigs. Changes in plating efficiency, saturation density and doubling time of transformed cells in medium containing 10% serum are not associated with progressive tumor growth. Formation of colonies in 0.35% agar appears to be the best index of tumorigenic potential. Frequently the capacity to grow as colonies in agar and produce tumors does not occur until 6-12 months following exposure to carcinogens such as benzo(a)pyrene, 3-methylcholanthrene or N-methyl-N'-nitro-N-nitrosoguanidine. Controls originally exposed to noncarcinogens including acetone, pyrene or anthracene do not form agar colonies or tumors after innoculation of 10^8 cells. Experience to date with guinea pig cells indicates that the changes in some growth parameters occur prior to expression of tumor growth. Formation of colonies in agar may more closely relate to tumorigenic potential than other commonly assessed properties of cells in culture.

5. Cell lines have been developed from clones isolated from transformed tumorigenic cultures originally exposed to benzo(a)pyrene, 3-methylcholanthrene, N-methyl-N'-nitro-N-nitrosoguanidine in vitro or to diethylnitrosoamine in the host-mediated in vivo - in vitro assay. These cloned cell lines like their parent transformed mass cell cultures all produce sarcomatous transplantable tumors in syngeneic guinea pigs after cells are inoculated intradermally, subcutaneously, intramuscularly or intraperitoneally. Cloned cell lines with differing morphology, growth characteristics and tumorigenicity have been isolated from the same parent mass culture of cells. Cloned tumor cell lines derived from cell cultures of the tumor resulting from each cloned cell line have also been obtained and preserved in liquid nitrogen. Each cloned cell line and its tumor line share similar morphological and growth characteristics consistent with the origin of the tumor cell line from the cloned transformed tumorigenic cells originally innoculated into the animal.

A cytotoxic assay measuring the amount of radioactivity released from tritiated thymidine labeled cells in the presence of phytohemagglutinin and mineral oil induced peritoneal exudate cells from normal adult syngeneic guinea pigs also shows that each cell line and its tumor cell line share a similar pattern of release which for all tumorigenic cell lines as a group is much greater than the quantity of radioactivity released from nontumorigenic transformed and nontransformed cell strains. In addition it has been observed that the skin reaction 24 hrs following intradermal inoculation of the cells into normal nonimmune guinea pigs is far greater to the tumorigenic cells. The in vitro peritoneal exudate cytotoxicity and in vivo

skin reactivity to nontumorigenic and to tumorigenic cell lines is being further explored to determine the value of these methods in comparison with growth of cells as colonies in agar as indices of the tumorigenic potential of cells and as additional means complementing the assessment of antigenic changes in the cells to assess alterations in the cell surface during the transition to neoplasia.

Significance to Biomedical Research and the Program of the Institute:

This project extends the current system of in vitro chemical carcinogenesis within the Cytogenetics and Cytology Section to an additional mammalian system. It also broadens the in vivo chemical carcinogenesis investigations within the Cellular Immunity Section to include in vitro chemical neoplastic transformation of strain-2 guinea pig cells. Development of this system will permit further opportunity to study chemical-target cell interaction without the complications inherent in in vivo analysis. In vitro chemical neoplastic transformation of strain-2 guinea pig cells will provide another assay system in addition to the Syrian hamster for screening of chemicals as potential carcinogens and broaden our understanding of chemical carcinogenesis at the cellular level through inspection of intracellular and cell surface alterations accompanying neoplastic transformation. Development of this new model offers an additional avenue for studying the process of carcinogenesis using a species with established tumor biology in which spontaneous transformation has not been seen and which possesses well defined immunological parameters.

Proposed Course of Project:

Conditions and methods will continue to be refined toward attaining optimal development of in vitro chemical transformation of strain-2 guinea pig cells as a reproducible, quantitative and reliable assay for potential chemical carcinogens. Conditions promoting the neoplastic growth of the transformed cells in syngeneic guinea pigs will be investigated. These will include in addition to irradiation the employment of immunosuppressive agents such as anti-lymphocyte serum and corticosteroids to determine if the absence of tumor formation is the result of immunological rejection of the transformed cells by the syngeneic host. Cell surface antigens of the transformed cells will be further defined to aid in this study and as part of the investigation of the mechanism of carcinogenesis at the cellular level. The establishment of the in vivo and in vitro antigenicity of the cells will be accompanied by specific definition of cell surface antigens including organ specific, Forssman, fetal or embryonic and tumor specific antigens. Examination will in turn proceed with extraction, isolation, purification and characterization of cell surface antigens seeking to define their relationship, if any, to chemical carcinogenesis as well as to tumor immunity. The elucidation of these relationships will provide further understanding of the mechanisms of chemical carcinogenesis at the cellular level and ultimately at the more complex levels of tumor establishment and immunity in the host animal.

Honors and Awards

Invited speaker, Twenty-fourth Annual Meeting of the Tissue Culture Association, Boston, Massachusetts, June 1973.

Serial No. NCI-4678
1. Biology Branch, Carcinogenesis, DCCP
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Relationships Between *In Vitro* Mutagenesis and Chemical Carcinogenesis

Previous Serial Number: None

Principal Investigator: Brian C. Myhr, Ph.D.

Other Investigator: Joseph A. DiPaolo, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.3
Professional:	1.1
Other:	0.2

Project Description

Objectives:

The ultimate objective of this project is to determine whether mutational processes are involved in the neoplastic transformation of mammalian cells by diverse chemical carcinogens. The accomplishment of this goal depends on the development of quantitative assays for mutational frequencies and the ability to perform both mutation assays and carcinogenesis assays with the same type of cultured cells. Accordingly, the current objectives are:

1. Establishment of the factors which influence the observed point mutation frequency to azaguanine resistance in Chinese hamster cells treated with several chemicals that are both mutagenic and carcinogenic.

2. Study of mutation frequencies in Chinese hamster cells resulting from successive treatment with two mutagens under conditions known to yield a marked enhancement of neoplastic transformation of Syrian hamster cells.

Methods Employed:

Chinese hamster cells (V79-4) have been utilized by other investigators to demonstrate the emergence of cells that survive in the presence of azaguanine after the cell population has been treated with known mutagens. The basis for survival is the lack of incorporation of azaguanine into the cellular DNA. This cellular alteration appears to be a mutational process, since

the lack of a functional enzyme, hypoxanthine guanine phosphoribosyl transferase (HGPRT), can often be demonstrated.

A number of factors are known to influence the observed frequency of azaguanine-resistant cells, including the elapsed time between the end of treatment and the introduction of azaguanine (the expression time), the concentration of azaguanine, and the total number of cells in the culture. These factors are being investigated with V79-4 cells in order to maximize the mutation frequenices observed after treatments with methylmethane sulfonate (MMS), N-acetoxy-2-fluorenylacetamide (AFAA), or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). In addition to the usual practice of treating and selecting in the same clonal population, another method has been developed wherein mass cultures are treated and the cells are then allowed to recover for several days before subsequent cloning and selection with azaguanine.

Major Findings:

1. The standard clonal assay procedure is generally inadequate for yielding maximum mutational frequencies and may underestimate some frequencies by 10-fold or more. The rapid decline in frequency after a peak in the 40-50 hour expression range is not seen when mass-treated cells are allowed to recover before cloning and selection.

2. The standard expression time of 42 hours is not nearly sufficient after treatments with MMS, AFAA, or MNNG. The mass treatment method shows that maximal expression requires at least 90 hours for a mild MMS treatment, while AFAA and MNNG require 150-180 hours.

3. Low concentrations of azaguanine allow increased numbers of survivors. The "effective concentration" of azaguanine depends on the serum component of the medium and may vary considerably from one lot to another. Dialysis of one lot of fetal bovine serum markedly reduced the spontaneous and induced mutation frequencies in the presence of 10ug azaguanine/ml medium.

4. The application of the mass treatment method to cells exposed to 500uM MMS followed by 7uM AFAA at varying times suggests the potent mutagenic action of AFAA may not be influenced by the prior action of the weak mutagen, MMS. This result contrasts with the enhancement by MMS of the neoplastic transformation of Syrian hamster cells by AFAA.

Significance to Biomedical Research and the Program of the Institute:

The significance of relating mutagenesis and carcinogenesis lies in providing a possible mechanism for chemical carcinogenesis and a basis for the etiology of human cancers that are caused by exposure to various chemicals and physical agents.

Proposed Course of the Project:

Complications arise in the interpretation of comparisons between the mutagenic action of a chemical on one cell type and its carcinogenic action on another kind of cell. Therefore, after the quantitative aspects of the mutation assay are firmly established, an attempt will be made to perform such assays with Syrian hamster cells, which can also be transformed by known mutagens.

SUMMARY REPORT

CARCINOGEN BIOASSAY AND PROGRAM RESOURCES BRANCH

July 1, 1973 through June 30, 1974

"Plans, develops and implements a coordinated research program to (1) test chemical and physical agents for carcinogenicity, by standardized bioassay methods, (2) establish the necessary methodology and logistics for the bioassay program, (3) develop and provide animal, chemical and information resources and data analysis to the Carcinogenesis Area, (4) monitor animal care and welfare in the intramural and collaborative contract programs, (5) develop and standardize selected models for carcinogenesis research, and (6) conduct studies aimed at determining the co-carcinogenicity of radiation with chemical agents."

The Branch was established in April 1973 in a reorganization of the Carcinogenesis Program. As a result, the Program and Data Analysis Unit, in the Office of the Director, was abolished and its activities along with those of the Bioassay Program, previously in the Experimental Pathology Branch, were consolidated into this new Branch. The Acting Head of the Program and Data Analysis Unit, Dr. Norbert P. Page, was appointed as Chief. No personnel transfers were involved in the reorganization. The reorganization and central management of the Bioassay Operations Segment under the responsibility of the Branch's staff has resulted in more efficiency in providing resources to the Bioassay Program.

The Branch staff is responsible for the collaborative contract program of two separate segments, (Bioassay Operations Segment and Information and Resources Segment). In addition, members of the staff also serve as Project Officers on contracts in the Biological Models and Lung Cancer segments. A discussion of these projects can be found in the narrative description for the segments. The Progress accomplished by the Branch in its direct activities is reported here under three main headings.

(1) Carcinogen Bioassay

During this first year the Branch has instituted a management system to provide for a more efficient Bioassay Program. The staff of this Branch with collaboration of other staff members from the Carcinogenesis Program has reviewed and implemented improvements in the design and standards of conduct of bioassay tests to detect carcinogenic hazards of chemical and physical agents, to be implemented by the collaborative program. This includes (1) identification and selection of chemical and physical agents for bioassay; (2) establishment of logistical capabilities for testing; (3) acquisition, characterization, and purification of these agents; (4) identification, development and selection of biological models for carcinogenesis bioassay including improved animal models and short-term bioassay procedures; (5) identification of carcinogenic activity of selected agents by the appropriate bioassay tests; (6) monitoring testing progress and performance; (7) development of a data

bank to include results of bioassay testing and information on use and characterization of the agents being tested; (8) analysis and evaluation of the test results; and (9) deciding on further action required of the tested agents.

Workshops and conferences have been held on protocol design, animal care standards and data dissemination. A continuing effort has been made to standardize and improve the protocols used for the bioassay of environmental carcinogenesis. Dr. Sontag has acted as Chairman of a working group for the revision of bioassay testing protocols. Dr. Squire has devoted considerable attention to standardizing the pathology techniques, protocol and reporting of results. The Branch Chief is Chairman of an Interagency Subtask Group on Carcinogen Testing which is reviewing the NCI revised protocol in relation to those of other agencies. The Branch has been asked to provide guidance to other Government agencies and industry on standards and protocol to be used in carcinogen bioassay testing. Capabilities have been developed to provide standard reference samples of many carcinogens for use in program activities.

A Chemical Selection Committee has been formulated to select chemicals for the testing program with Dr. E. Weisburger as Chairman and Dr. M. Litwack as Executive Secretary. Main criteria used for selection are production and exposure data and chemical structure. To assist the selection committee information dossiers, including previous toxicity, metabolism studies, stability, usual exposure modes, cost and availability, are prepared by the Branch's information activities. Nominations of chemicals are received from various Government agencies through the Scientific Coordinator for Environmental Carcinogenesis. The Branch is responsible for the preparation of critical reviews of the results of bioassay testing. This requires evaluating the conduct of the experiments as well as an analysis of the results. Assistance is obtained from the Field Studies and Statistics Program in the statistical analysis of the data. As the results of these experiments can be important in establishing public policy on the use of the agents tested, release of information is made following a critical evaluation of the results and with the approval of the Director of the Carcinogenesis Program. The Scientific Coordinator for Environmental Carcinogenesis is responsible for coordinating release of information to other Government agencies.

Due to the large administrative burden, a Prime Contract has been awarded to assist in the management of the Bioassay Program. This should facilitate the orderly planning of bioassay testing and the coordination of animal, chemical, information and laboratory resources that are required.

(2) Information Activities

Develops, implements and maintains information systems to gather, catalogue, and disseminate information to the Carcinogenesis Program's scientific and administrative staff. Performs literature searches and compiles information as a staff support activity.

The Carcinogenesis Bioassay Data System (CBDS), whose design was initiated by Drs. U. Saffiotti, J. A. Peters and J. A. Cooper (OAD), then developed and established by members of this Branch in collaboration with the NIH

Division of Computer Research and Technology (DCRT), is now used to collect, monitor and store experimental data and results from bioassay operations. The system is designed for the complete and selective recall of bioassay data. It is now in full operation with new bioassay experiments being entered as they are initiated. Data from 20 contractors are now on file with several other contractors preparing data for entry into the system. During the current year the staff expanded the system to permit the analysis of data using those methods best suited to the interpretation of carcinogenesis data. In addition, chemical and bioassay contract information sub-systems are maintained which list data on materials presently under test or projected for study, date initiated, etc.

The Branch is responsible for the publication of the Survey of Compounds Which Have Been Tested for Carcinogenic Activity (PHS Publication No. 149) and Carcinogenesis Abstracts. PHS 149 is produced in a series of volumes which represent a standard reference data base in the field of chemical carcinogenesis bioassays. Volumes are now available for literature published up to 1971. A volume covering 1972-1973 is now being processed. Carcinogenesis Abstracts, is a monthly publication which provides the scientific community with current abstracts on all aspects of carcinogenesis research.

Carcinogenesis information searches were conducted as requested by Carcinogenesis staff utilizing data bases such as the Smithsonian Information Exchange, MEDLARS, National Library of Medicine Toxicology Information Program, and the Selective Dissemination System of the USDA.

Due to the rapidly expanding need for information tabulation and literature retrievals, an information resource support contract has been awarded, to provide general information support to all of the Carcinogenesis Program. A conference on "Data Dissemination in Carcinogenesis" was held in Williamsburg, Virginia on January 30 - February 1, 1974, to discuss methods of storing and disseminating the large amount of complex data generated by Carcinogenesis research programs. Staff collaborated with the OD, NCI, in the development of the International Cancer Research Data Bank and of the NCI Management Information System.

(3) Animal Resources and Care

The Branch is responsible for the procurement of animals for the intramural and collaborative contract programs, and assures compliance with the animal welfare laws and HEW Guide for Care and Use of Laboratory Animals. It also assists Carcinogenesis staff with animal health and husbandry problems and plans animal facility modifications, plans and supports the development of animal models for cancer research as well as collecting and making available baseline information on laboratory animals.

The Branch recognized a need to develop a more extensive program to meet the animal requirements of the Carcinogenesis Program and is assisting the program directors in planning their animal needs as far in advance as possible, coordinating these needs with the animal suppliers and providing information on the characteristics and availability of laboratory animals. Assurances of compliance with the Animal Care and Welfare standards are

obtained for both contract and intramural programs. Dr. N. Page is currently Chairman and Dr. T. Cameron is a member of the NCI ad hoc Group appointed for that purpose. The main animals used in the bioassay program are Fischer/344 and Osborne-Mendel rats and B6C3F1 hybrid mice. Supplies of these animals, in the past, were procured primarily through cooperation of the Animal Supply System of the Division of Cancer Therapy by transfer of funds. SPF colonies have now been developed at the Frederick Cancer Research Center to supply the Large-Scale Bioassay Project at the Frederick Cancer Research Center and hopefully to other bioassay contractors. Colonies of strain 2 and 13 Guinea pigs have also developed to the point of meeting current program needs. Stock animals for the Frederick Cancer Research Center breeding colonies were supplied by the NIH Veterinary Resources Branch (DRS).

Considerable effort has gone into improving animal care in the Program. Along this line a workshop on "Standards for Successful Long-Term Rodent Studies" was sponsored by this Branch and by the Information and Resources Segment in conjunction with the Institute of Laboratory Animal Resources (NAS/NRC). The proceedings will soon be published as an NAS/NRC document.

An outbreak of Ectromelia ("mouse pox"), inadvertantly introduced into Building 37 in January 1974, through a transplantable tumor shipped from abroad, was effectively contained by quick action on the part of the staff, which prevented a widespread epidemic from occurring and destroying the entire animal colony in the building. This episode has dramatically emphasized the lack of adequate animal facilities for the laboratories in Building 37. This Branch has been charged with preparing a plan for remedying this serious deficiency.

(4) Other Activities

The Branch Chief functions as the radiation biologist for the Carcinogenesis Program. A limited contract program in radiation carcinogenesis has been developed on the interactions of radiation and chemicals, in the induction of lung and breast cancer and to determine the effects of a reduction of stratospheric ozone by supersonic transports on the incidence of UV-induced skin cancer.

Serial No. NCI-4564
1. Carcinogen Bioassay and Program Resources Branch, Carcinogenesis, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Carcinogen Bioassay Program

Previous Serial Number: None

Principal Investigator: Norbert P. Page, D.V.M.

Other Investigators: James Sontag, Ph.D.; Thomas Cameron, D.V.M.; Sidney Siegel, Ph.D.; Marcia Litwack, Ph.D.; Herman Kraybill, Ph.D.; Robert Squire, D.V.M., Ph.D.; Elizabeth Weisburger, Ph.D.; John Cooper, Ph.D.; Umberto Saffiotti, M.D.; John Gart, Ph.D.; Thomas Fears, Ph.D.

Cooperating Units: Collaborating Scientists and Advisors of the Carcinogenesis Program; Field Studies and Statistics Program

Man Years:
Total: 4.0
Professional: 2.5
Other: 1.5

Project Description

Objectives:

To coordinate and manage the Carcinogen Bioassay Program which identifies and evaluates carcinogenic hazards of chemical and physical agents in the environment.

Methods Employed:

Standardized long-term animal bioassay tests are utilized to assess for carcinogenicity. Considerable planning is required to procure and coordinate the animal, chemical, information and laboratory resources required for an efficient and quality operation. Details of specific methods employed are provided in "Project Reports" for the particular activity.

As resources and funds are not available to test all chemicals introduced into the environment, a judicious selection must be made. A Chemical

Selection Committee selects chemicals on the basis of human exposure and production data, and chemical structure. Dossiers for each chemical are prepared which include information on toxicity, metabolism, stability, usual exposure modes, cost, availability, etc. To be responsive to all Government or National interests, representatives from other agencies provide nominations of chemicals for testing. Once selected, the chemicals must be procured, often specifically synthesized, purified, and analyzed. Stability and volatility measurements are made of bulk chemicals or as prepared for administration, e.g., in feed. The majority of testing is by contract laboratories having the required facilities and scientific staff.

In addition to providing for testing facilities and chemical resources, animal needs must be planned in sufficient quantity and quality for the studies to commence on an orderly schedule. The main animals used in the bioassay program are the Fischer/344 and Osborne-Mendel rats and B6C3F1 hybrid mouse (C57B1/6 x C3H). Most of the animal needs are supplied by the Frederick Cancer Research Center. Due to the required period for expanding animal production colonies, needs must be forecast several months in advance. An "Animal Supply" Information System is used to provide future animal requirements to the animal farm. Animals on long-term tests are maintained under strict environmental surveillance to prevent the introduction and spread of infectious diseases that can quickly decimate rodent colonies. Contractors are required to comply with Animal Welfare Laws and the DHEW Publication 73-23, "Guide for the Care and Use of Laboratory Animals." Letters of compliance are required by the contracting office. Considerable effort has gone into improving animal care in the Program. Along this line a workshop on "Standards for Successful Rodent Studies" was sponsored by this Branch and by the Information and Resources Segment in conjunction with the Institute of Laboratory Animal Resources (NAS). The proceedings will soon be published as a National Academy of Sciences Document.

Monitoring of the quality and progress of individual studies are through visits and reviews, the Carcinogenesis Bioassay Data System (CBDS), and routine progress reports.

It is important that bioassay data be recorded, analyzed and reported properly. Currently, this is through two main means. For studies initiated in the past 1 1/2 years, data are presented by use of a computerized data system known as the Carcinogenesis Bioassay Data System (CBDS). Data from earlier studies are reported by progress, special or final reports.

The CBDS provides for the collection, monitoring and reporting of data or information about bioassay studies. It is designed for the complete or selective recall of bioassay data. The system is now in full operation with new bioassay experiments entered as they are initiated. Data from about 20 contractors are now on file. A major effort this year was the expansion of the system to permit for the analysis of the data.

In addition to the main data system, chemical and bioassay contract information subsystems are maintained which list data on material presently under test or projected for study, date initiated, etc.

All contractors are required to provide an annual detailed progress report four months prior to termination date. Brief but informative interim progress reports are required every four months except at time of submission of the annual report. Contractors are encouraged to submit the results of their testing to scientific journals having wide dissemination to the scientific community. Journals will not accept bioassay data due to the bulk and often negative results. To discuss methods of storing and disseminating the large amount of complex data generated by Carcinogenesis research programs a conference on "Data Dissemination in Carcinogenesis" was held in Williamsburg, Virginia on January 30 - February 1, 1974.

Some contractors do not possess their own in-house capability for properly analyzing the results of their studies; neither does the Branch. For this reason, statisticians from other areas of the NCI have been asked to assist in this area. The members of the Field Studies and Statistics Program (DCCP) possess in-house capabilities to accomodate most of the needs in this area.

It is important that the results of these tests be properly tabulated and analyzed prior to release to the public or other agencies. For this reason all release of bioassay data must be reviewed and approved prior to contractor release. The Scientific Coordinator for Environmental Carcinogenesis is responsible for discussing the results with other agencies. Where necessary, panels of experts are convened to review the pathology lesions or data produced in the Bioassay Program.

Major Findings:

A large expansion of the program took place in the past two years; such that approximately 450 chemicals are now on test. This includes a large number of pharmaceutical agents, pesticides and other agricultural chemicals and industrial chemicals to which man is exposed. A number of these chemicals, especially pharmaceuticals and pesticides, have been found to possess strong carcinogenic activity. During this current year several other long-term experiments have terminated and are awaiting completion of pathological examination and data analysis.

Significance to Biomedical Research and the Program of the Insititute:

Protecting the public from chemical and physical carcinogenic hazards and their effects has been clearly recognized as an urgent and essential task in our society and a high national priority for this mission has been indicated by the National Cancer Act, Congress and the Administration. One of the major program activities is the identification of carcinogenic activity of selected agents by bioassay testing. The results of this testing program are utilized in the establishment of public policy by regulatory agencies in regard to utilization of the test agents. It is estimated that several hundred thousand different chemical entitites are present in the environment. With modern technology the annual accrual rate is increasing the environmental burden at an astounding rate. Many

of these factors have not yet been assayed for carcinogenicity. It is important that such information be obtained if the current epidemic of cancer is to be abated. There is little doubt that environmental agents play a major role in the causation of cancer. It is anticipated that the results from this program may prevent the unintentional exposure of man to carcinogenic materials.

Proposed Course of Project:

The expansion of this program was temporarily halted during the past year to allow for the development of animal and chemical resources. These resources are now functioning smoothly and capable of meeting all anticipated needs of the program. Additional agents have been identified by the Chemical Selection Committee. While Budgettary restraints will not permit all those of high priority to be tested; it is expected that a couple new contracts will be awarded in the next year.

Many chemicals will reach the end of their testing phase in the next year resulting in a heavy workload in pathology, data analysis and reporting. A standard reporting format will be implemented during this next year.

Additional studies are needed to improve the methodology of testing. Less expensive and shorter screening methods than the currently used two to three-year in vivo system are needed. Many studies in the past have suffered from latent toxicity to the continuous administration of chemicals at what was thought to be a "maximum-tolerated-dose" (MTD). Research is needed to better predict a tolerated level for long-term treatment. One of the major changes in the bioassay protocol is to extend the period for the subchronic range-finding tests. A critical review of the protocols in use is now taking place. Particular attention is being given to the following areas: (a) chemical quality and distribution, (b) animal quality and surveillance, (c) control of physical and chemical environment, including personnel hazards, (d) experimental design, (e) pathology, (f) data collection and analysis, and (g) reporting of results.

There is an urgent need to standardize the conduct of pathology techniques and reporting. The SNOP-code was developed for human pathology and needs refined for use with animal experimental results. More tissue sections of interesting tumors will be provided to the Registry of Experimental Cancers. The adequacy and quality of the pathology staffs varies somewhat in the contract program and it may be desirable in some cases to provide for training of technicians, etc., to assure quality performance. The recently-created Tumor Pathology Section, which incorporated the Registry of Experimental Cancers, is responsible for the pathology activities. Assistance is provided by Pathology Support Contracts.

To effectively manage and monitor the Bioassay Program requires expertise in various disciplines including research management, animal care, chemistry, toxicology, biometry, pathology, information handling, and fiscal operations.

While the Carcinogen Bioassay and Program Resources Branch and cooperating groups in Carcinogenesis possess several of these disciplines, the need for more staff is urgent in view of the projected further expansion of bioassay and carcinogenesis activities. Unfortunately, it does not appear at this time that these personnel requirements can be met. To partially offset this setback, it is hoped that certain functions can be accomplished by a Prime Contract to manage part of the Bioassay Program, as well as through other contract to support pathology and information activities.

Honors and Awards

Chief, Carcinogen Bioassay and Program Resources Branch

Director, Bioassay Operations Segment, Carcinogenesis, NCI

Chairman, NCI ad hoc Group on Animal Welfare

Member, Division of Cancer Cause and Prevention Interprogram Group, NCI

Member, Civil Defense Committee on Vulnerability of Food Crops and Livestock Production to Fallout Radiation

Member, Carcinogensis Contract Program Management Group

Member, Interagency Collaborative Group on Environmental Carcinogenesis

Member, Information and Resources Advisory Group, Carcinogenesis, NCI

Chairman, Interagency Sub-task Group on Carcinogen Testing

Member, National Cancer Panel Committee to Assess Cause and Prevention in Cancer Control Program

Chairman, Evaluation Group to Select Carcinogenesis Pathology Support Contracts

Chairman, Evaluation Group to Select Bioassay Prime Contract

Member, Workshop on Protocol for Carcinogen Testing

Serial No. NCI-4539
1. Carcinogen Bioassay and Program Resources Branch, Carcinogenesis, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Coordination of Information Activities and Data Dissemination for the Carcinogenesis Program

Previous Serial Number: Same

Principal Investigator: Sidney Siegel, Ph.D.

Other Investigators: Norbert P. Page, D.V.M.; T. D. C. Kuch; Umberto Saffiotti, M.D.; John A. Cooper, Ph.D.; Thomas P. Cameron, D.V.M.; Marcia D. Litwack, Ph.D.

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.5
Other:	0.5

Project Description

Objectives:

To develop, implement and maintain systems which gather, catalogue and disseminate data and information relevant to the Carcinogenesis Program.

In so doing, create, maintain and/or search data bases which will enable scientists and scientist administrators within the broad spectrum of disciplines comprising carcinogenesis research to be made aware of data and information relevant to their own efforts and responsibilities.

Methods Employed:

While much of the data and information gathering and dissemination is performed by Branch personnel, the needs far exceed in-house capability, and contractors are employed to perform specific tasks. A full listing of these tasks is available in the section describing the Information and Resources Segment. Activities used to respond to program needs can be generally grouped as regularly occurring publications which gather abstract and/or extract relevant data and information from the scientific literature or as information research projects. The research projects, in various stages of evolution, have enabled Program to

logically explore the approaches needed to define, uncover, collate, evaluate, store and retrieve data and information research, research-administrative and regulatory responses on problems which exist now and are projected to exist for the Carcinogenesis Program.

Coordination of these existing, experimental and projected nodes of information activity is required for efficient and effective responses to Program's information problems. Among these problems are concerns of general toxicologic phenomena, carcinogeneicity, mutagenicity, metabolism and the production, distribution and exposure of materials to segments of the human population.

Major Findings:

Literature extraction activity required of the contractor for Carcinogenesis Abstracts and PHS #149, Survey of Compounds Which Have Been Tested for Carcinogenic Activity, indicates that there has been a significant and stable increase of program relevant information in the various disciplines which comprise carcinogenesis research.

Significance to Biomedical Research and the Program of the Institute:

Development, implementation and monitoring of research programs requires a continual flow of information, which must be ordered or indexed in a manner so data and information generated can be searched or manipulated in a logical and useful manner. Once data bases are established, criteria on reliability of the data can be used as a qualification filter for inclusion or as a method for annotating what is retained. Information projects which are now on-going and those ready for implementation are designed to meet scientific and scientific-administrative needs of the Carcinogenesis Program.

Proposed Course of Project:

The project will continue its formal information publications as in the past. Informal surveys indicate a high level of demand by the scientific and scientific administrative community for their continuation.

As a result of positive mandate given the Carcinogenesis Program in Williamsburg, Virginia, (January 1974) this subset of the information program will start to explore the possibility of developing, on a modular basis, the capability of disseminating data generated by or of interest to carcinogenesis researchers.

The project will continue to be sensitive to the general and specific information needs of the Carcinogenesis Program. Information systems to be generated will be so structured that they will be able to respond to the ever-changing profile of research. The systems will be designed to store new and different data with more conventionally formatted data of the past while still having the ability to search and retrieve both kinds of information.

Honors and Awards

Member, Information and Resources Segment Core Working Group

Session Coordinator at the International Cancer Research Data Bank Planning Conference at Airlie House, Warrenton, Virginia, May 22-24, 1972

Member, Review Panel to Evaluate Proposals for the National Cancer Program Cancer Information System (NCP-CIS), February 1973

Staff Member, Bioassay Operations Segment

Member, Review Panel to Evaluate Proposals for Establishment of a Carcinogen Bioassay Prime Contractor

Co-organizer and Participant, Data Dissemination Conference for the Carcinogenesis Program, (Interrelationships between the Carcinogenesis Program and the concepts, techniques and problems of data dissemination), Williamsburg, Virginia, January 30 through February 1, 1974

Carcinogenesis Program Representative at the International Agency for Research on Cancer Meetings concerning, "Organochlorines" and "Hormonally Active Steroids", held in Lyon, France October 1973 and February 1974

Member, Interagency Collaborative Group on Environmental Carcinogenesis

Member, Toxicology Information Subcommittee of the DHEW Committee to Coordinate Toxicology and Related Programs

Serial No. NCI-4699
1. Carcinogen Bioassay and Program Resources Branch, Carcinogenesis, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Carcinogenesis Bioassay Data System

Previous Serial Number: Same

Principal Investigators: Norbert P. Page, D.V.M.; John A. Cooper II, Ph.D.; T. D. C. Kuch; Mary S. Linhart

Other Investigators: Sidney Siegel, Ph.D., Ursula Evans

Cooperating Units: Data Management Branch, Division of Computer Research and Technology, NIH; Wolf Research and Development Corporation; Field Studies and Statistics Program, NCI

Man Years (NCI):

Total:	2.65
Professional:	2.45
Other:	.2

Project Description

Objectives:

To develop, implement, and maintain a comprehensive information system for collection, storage, manipulation, retrieval, and analysis of data pertaining to carcinogenesis bioassay studies. In so doing, to create and maintain a file of data about (a) substances studied as part of the Chemical Carcinogenesis Program, (b) experiments and related experimental design in bioassay studies, (c) diagnoses of animal deaths from suspected carcinogenic agents on trial, and corresponding control groups.

Methods Employed:

Standard taxonomic, system-operational, systems design, computer programming, and statistical computing techniques, as well as the NIH/DCRT hardware and software environment have been used in the development of the system.

A chemical data subsystem has been merged into the CBDS. This contains data such as these, on substances under investigation:

Chemical Abstracts Service (CAS) name
Synonyms (common and trade names)
CAS number
NCI chemical number
Chemotherapy (NCI) chemical number
Wiswesser Line Notation
Molecular formula
Molecular weight
Category and subcategory of use (e.g., pesticide:rodenticide)
Information on bioassay contractors using this substance

Organization of the CBDS: The Carcinogenesis Bioassay Data System comprises these subsystems:

(1) Bioassay Contractor Subsystem - The activities of contractor pathologists and clerical staff in documenting the design and results of experiments in testing possible carcinogenic agents in animal systems, communicating these designs and results in standard form to the NCI through the Operations Subsystem.

(2) Operations Subsystem - The activities of data transcription and systems operation personnel in obtaining completed forms from bioassay contractors; checking them for accuracy and completeness; logging their progress through the system; microfilming them; keypunching and key-verifying input documents corresponding to data forms; submission of computer jobs representing new or replacement data for entry into the CBDS data base; and related functions.

(3) Computer Subsystem - The activities of computer systems designers and programmers in designing, implementing, and maintaining the CBDS data base and the CBDS system of computer programs to manage that data base; and generating preliminary management and scientific output reports.

(4) Statistical Analysis Subsystem - The activities of computer programmers and computational statisticians in analyzing chronic toxicity and carcinogenicity data of CBDS test animals, using standard statistical methods. We have been fortunate to enlist the consulting services of non-Carcinogenesis Program professionals including Dr. John Gart, FS&S; Dr. Hugh Pettigrew, FS&S; and Robert Baird, DCRT.

Major Findings:

The implementation stage of the Computer Subsystem has been successfully completed. Maintenance will continue throughout the life of the project, as continual adjustments must be made to ensure that bioassay contractor data remains fully compatible with data already in the

system and to modify the data system such that new studies, which may have been initiated with divergent experimental designs, can furnish data to the CBDS. The data base, stored on magnetic media, has now grown to approximately 110,000 records (est. 6/74). While some experiments are nearing completion, allowing data from them to be fully analyzed in the statistical analysis subsystem, others are in their early stages. Computer Subsystem output, in the form of summary reports and simple graphs, have been produced during the past year.

Significance to Biomedical Research and the Program of the Institute:

Because of the size, diversity, and complexity of the Carcinogenesis Bioassay Program, it is essential that a method be available to manage and make optimal use of the information generated by its various activities. The CBDS will provide this and will in addition contribute to uniformity of reporting results of bioassay studies and to standardization of chemical and tumor nomenclature. It will also provide for routine rapid evaluation of bioassay studies, including comparison of results from multiple sources, and for ready access to summary data for statistical analysis.

Proposed Course of Project:

New bioassay studies will be entered into the system as they are initiated. During FY75, these activities will include, by subsystem:

(1) Bioassay Contractor Subsystem - Continue to integrate new and existing contractors into the CBDS. Retrospectively incorporate older, non-CBDS bioassay studies into the CBDS.

(2) Operations Subsystem - Continued operational responsibilities for data conversion, data logging, inputting of data into the Computer Subsystems, and microfilming of documents for the source document file. The contractor for this subsystem has proved responsive and has functioned in an acceptable manner when given opportunity to interact directly with the bioassay contractor personnel (including pathologists). He has been given greater responsibilities in this area.

(3) Computer Subsystem - System development has now ended, and continued modification and maintenance of this Subsystem will be continued in the future, using personnel from the DMB, DCRT.

(4) Statistical Analysis Subsystem - Preliminary development of statistical reports has been done, and the more obvious statistical analyses are being made on animal groups as experiments on them are being completed. During FY75 more sophisticated statistical work will be planned and executed, in collaboration with the DMB, DCRT, and the Field Studies and Statistics Program.

Honors and Awards

John A. Cooper II, Ph.D.

Member, Panel on Strategy of Carcinogenesis Testing in the Plan in Development of New Drugs, Conference on Carcinogenesis Testing in the Development of New Drugs organized by the National Academy of Sciences and held May 23-25, 1973

Participant, Working Group on the Evaluation of Carcinogenic Risk of Chemicals to Man, International Agency for Research on Cancer, World Health Organization on June 18-25, 1973

Speaker, "Discussion of the Federal Standards for the Handling of Chemical Carcinogens" at the Fourth Joint Working Conference, DCT, NCI on November 21, 1973

Speaker, "Discussion on Stanford Research Institute Activities (Contract NO1-CP-33285)" at the Meeting of Coordinating Committee for U.S. Commission of the European Communities Environmental Cooperation on February 7, 1973

Director, Information and Resources Segment, Carcinogenesis, DCCP, NCI

Member, Cause and Prevention Executive Staff, DCCP, NCI

Member, Carcinogenesis Contract Program Management Group, DCCP, NCI

Member, Carcinogenesis Intramural Program Group, DCCP, NCI

Member, Chemical Selection Committee, Carcinogenesis, DCCP, NCI

Publications

Linhart, M.S., Cooper, J.A., Martin, R.L., Page, N.P., Peters, J.A.: Carcinogenesis Bioassay Data System. J. Comp. Biomed. Res. (In Press).

Linhart, M.S., Martin, R.L.: Carcinogenesis Bioassay Data System. U.S. DHEW, PHS, NIH, DCRT. NIH Technical Report No. 10, 1973.

Serial No. NCI-4541
1. Carcinogen Bioassay and Program Resources Branch, Carcinogenesis, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Animal Resources

Previous Serial Number: Same

Principal Investigators: Thomas P. Cameron, D.V.M.

Other Investigator: Norbert P. Page, D.V.M.

Cooperating Units: Animal Farm, Frederick Cancer Research Center; Carl Hansen, Ph.D., Genetics Unit, Rodent and Rabbit Production Section, VRB, DRS; Samuel Poiley, Mammalian Genetics and Animal Production Section, DCT

Man Years:

Total:	4.3
Professional:	1.3
Other:	3.0

Project Description

Objectives:

(1) Plan and effect procurement of animals used in the Carcinogenesis intramural and contract programs; (2) Assure compliance with the Animal Welfare Laws and the HEW Guide for Care and Use of Laboratory Animals; and (3) Plan and support the development of animal models for cancer research as well as to collect and provide information on laboratory animals.

Methods Employed:

The number of animals required for the various aspects of the Carcinogenesis Program are estimated as far in advance as possible by the program staff and the staff of the various contracts. These needs, for the first four months of this fiscal year, were conveyed to the Mammalian Genetics and Animal Production Section, DCT, for supply from the NIH colonies or contract animal suppliers. This mechanism was supported by funds from the Carcinogenesis Program that were transfered to DCT.

From November 1, the major portion of the animal resource needs of the program have been supplied by the Animal Farm of FCRC. The breeding stock for this operation were, and continue to be, supplied by the Genetics Unit of the Rodent and Rabbit Production Section, VRB, DRS.

A well organized system of projecting, ordering and reporting animal deliveries, of various species and strains, has been developed and coordinated between the staff of Wolf/EG&G and the production staff of the Animal Farm, FCRC. This system embraces three components; a six-month projection of needs by the contractor components of the Bioassay Program, a monthly requisition of actual needs, and a weekly report of receipts.

Staff members have overall responsibility for the animal supply and husbandry of the Bioassay Program. This responsibility includes offering guidance on methodology and caging. During this year, a considerable amount of time has been expended in consulting on new construction and extensive renovations both in bioassay contracts and at FCRC.

It has become apparent that there was no one adequate document available that could guide contractors or prospective contractors in the proper maintenance of laboratory animals, renovation of present facilities or construction of new buildings. If such a document were to be made available, it could serve to discourage bioassay contract proposals that were clearly inadequate from the standpoint of animal husbandry. This would save a great deal of proposal-reviewing effort by the staff members and, more importantly, save the proposers a great deal of money and effort in presenting proposals that would have no possibility of being seriously considered because of gross deficiencies in buildings, equipment, methodology or personnel.

To remedy this lack, a task order was attached to an NIH contract with the Institute of Laboratory Animal Resources, National Research Council/National Academy of Science, for the purpose of developing "Standards for Successful Chronic Rodent Studies (Mice, Rats and Hamsters)". A committee was formed by ILAR, based upon a workshop sponsored and organized by the Branch staff and held in April 1973. A suitable standard will be drawn up by the committee by the end of the contract year and published by the NAS.

Considerable effort has been made to develop satisfactory animal models for cancer research. Contract support has been continued to Dr. Ulrich Mohr, Medizinische Hochschule, Hannover, West Germany, for the development of the European Hamster as an animal model primarily for lung cancer research. Preliminary efforts have begun to increase the Strain 13 guinea pig colony at FCRC. A high incidence of pancreatic cancer has been chemically induced in that strain and a greatly increased demand is anticipated. A need for aged rats

(18-24 months) has been defined in certain areas of the Carcinogenesis Program and it is expected that a contract mechanism will be developed for their production and distribution.

Two other areas have required a considerable expenditure of time by staff members. By the direction of the Director, NCI, a search has been made for suitable candidates for a position tentatively titled, Director, Intramural Animal Care, NCI. With the aid of an outside advisor, three candidates, each a Diplomate of the American College of Laboratory Animal Medicine, were selected. Each candidate presented a seminar to the Directorate of NCI concerning his qualifications and concepts of general laboratory animal care. The selection of a director now rests with the staff of the Director, NCI.

A highly infectious, fatal disease of mice, Ectromelia (also called mouse pox) was inadvertently introduced into Building 37 by the importation of tumor-bearing mice from University Hospital, London. Staff members were designated to act as a focal point for control of this potential epizootic.

Major Findings:

The main thrust of this resource area has been to produce sufficient numbers of animals of extremely high health status for the bioassay program. This goal has been achieved with the establishment of the required breeding colonies at FCRC and the attainment of a level of production that is considered adequate for program needs for the forseeable future. The production level of effort for the Animal Farm, FCRC, reached 6000 per month each of F344 rats and B6C3F1 mice by the beginning of November 1973. It is expected that this level of effort will be maintained indefinitely until program needs dictate an adjustment of colonies. Other strains, with high priority for the program's needs, have been established at FCRC. For instance, the production of Strain 2 and Strain 13 guinea pigs - both virtually unattainable from other areas of the Cancer Program or any commercial sources - has reached the level where they are occassionally in excess of intramural demands and can be shipped to NCI contractors for the continuation and expansion of their directed contract efforts.

The establishment of the European Hamster as an animal model continues at a satisfactory rate. The colony now numbers approximately 500 animals and is now in the F4 Generation. The first beginnings have been made in the establishment of an inbred strain; this effort has now progressed beyond the F2 generation. This contract has produced one manuscript explaining the husbandry and breeding of this animal in the publication of the American Association of Laboratory Animal Science. In addition, in review is an excellent, well-illustrated anatomical atlas of the European Hamster. It is projected that this atlas will be published through the cooperation of the Government Printing Office.

Significance to Biomedical Research and the Program of the Institute:

Biomedical research to a very large degree depends upon the selection of the proper animal model, the supply of sufficient numbers of healthy animals to satisfy the protocols, and the correct care of the animals while under test. These factors are especially important to the extensive Bioassay Program in which animals are held for the greater part or all of their life span to study the carcinogenic influences of certain compounds. Animal deaths, from non-experimental influences midway or later in this type of study, represent a significant loss in time and money.

It is essential that the staff supply the proper quality and numbers of animals to the program and monitor their care both in the intramural and extramural programs. This is done through the contract mechanism (as project officers or resource advisors) for the extramural effort and as members of the Ad Hoc Committee for NCI Animal Welfare in the case of the intramural components.

Likewise, the development of new or more sensitive animal models in cancer research is a vital concern to program. As new animal needs are identified and demands are created, the supply mechanism must be created so that biomedical research progresses.

Proposed Course of Project:

The project will continue as defined. The intramural and extramural programs will be serviced in the areas of animal supply, monitoring of animal husbandry practices, and the development of needed animal models in collaborative efforts.

A need has been defined to establish an additional source of animals for the program in order to insure continuity of supply in the event that a catastrophe, epizootic or mechanical in nature, interferes with the animal production of the Animal Farm, FCRC. To this end, a Request for Proposal has been advertised for the establishment of a gnotobiote based rodent production contract. This operation is designed to eventually produce, by the end of the second year, numbers of animals roughly equal to one fifth or one sixth of the FCRC effort. In the event of a problem with the Animal Farm, FCRC production effort, those programs of the highest priority could be supported by this contract. Animals of the species, strains and stocks that are required in relatively small numbers will continue to be supplied by the DCT program.

It is anticipated that the effort required to supply the in and out-house programs will be considerably reduced by the successful implementation of the FCRC Animal Farm effort. Likewise, the input of the staff into the day-to-day animal problems of the Bioassay Program will be minimized by the operation of the Bioassay Prime Contract. The time

made available will be devoted increasingly to the collection and dissemination of information on laboratory animals and the development of new animal models for cancer research.

Honors and Awards

Member, NCI ad hoc Group on Animal Welfare

Chairman, Program Committee, Annual Seminar National Chapter Area Branch, American Association for Laboratory Animal Science

Program Chairman, 25th Annual Session of the American Association for Laboratory Animal Science

Publications

Cooney, D. A., Homan, E. R., Cameron, T. P., and Schaeppi, U.: Measurement of 4-(^{14}C)-L-asparagine in body fluids and tissues: methodology and application. J. Lab. Clin. Med. 81(3): 455-466, 1973

Cameron, T. P., Kasel, J. A., and Couch, R. B.: Persistence of antibody to envelop antigens of A-Equi2 Neq2 Virus in ponies after infection in vaccination. Proc. Exp. Bio. Med. (In Press)

Serial No. NCI-45 40
1. Carcinogen Bioassay and Program Resources Branch, Carcinogenesis DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Radiation Carcinogenesis Program

Previous Serial Number: Same

Principal Investigator: Norbert P. Page, D.V.M.

Other Investigators: Douglas H. Janss, Ph.D., Experimental Pathology Branch, Carcinogenesis; D. Jane Taylor, Ph.D., Head, Experimental Biology Project Section, DCBD

Cooperating Units: Atomic Energy Commission; Bureau of Radiological Health, FDA, HEW; Environmental Protection Agency

Man Years:
Total: .50
Professional: .40
Other: .10

Project Description

Objectives:

(1) To develop a program directed toward the understanding of interactions and potential synergism of radiation with chemical agents. (2) To assess the effects of increased UV radiation from depletion of stratospheric ozone on skin carcinogenesis.

Methods Employed:

The majority of this program is devoted to the study of interactions of radiation with other environmental agents. A considerable effort is made to avoid duplication of research underway by other agencies.

The Principal Investigator remains informed of research support by other agencies by participating in reviews or scientific evaluations of on-going or proposed research programs. As limited facilities are available to expand the intramural research program, this area is being developed mainly through the collaborative contract program.

To assess for interactions or synergism, groups of animals are exposed to radiation and chemicals alone or simultaneously. Following this they are maintained for their normal lifespan to compare the relative incidence and type of tumors induced. The lung cancer studies use particles of alpha-emitting Polonium-210 and/or Benzpyrene-hematite. The interaction of smoking and radiation is assessed by epidemiological and exfoliative cytology studies of uranium miners. Estrogenic hormones, and radiation (X and neutron) are used to study interactions in the induction of breast cancer.

To assess the effects of depletion of stratospheric ozone on UV-induced skin carcinogenesis, an ozone chamber is being constructed to produce various thicknesses of ozone that filter the solar-simulated UV spectum. Hairless mice are exposed to the UV radiation and maintained for the development of skin tumors.

Major Findings:

Summaries of on-going radiation carcinogenesis research have been obtained through the Science Information Exchange (SIE) of the Smithsonian Institute. Contacts have been made with other Government agencies to receive annual progress reports as well as other information pertaining to radiation carcinogenesis research. A close liaison has been established with the Atomic Energy Commission in this regard.

While much research has been conducted on the hazards of radiation, the mechanisms by which radiation exposure can result in cancer and of the interactions of radiation with other agents remains largely unknown. The results from these studies indicate that radiation acts synergistically with chemials in inducing breast and lung cancer in animals. From the uranium-miner studies a similar relationship appears to exist in man.

Significance to Biomedical Research and the Program of the Institute:

The National Cancer Institute is concerned with carcinogenesis regardless of the etiological agent. Within the past few years the Institute has not supported a large program in radiation carcinogenesis mainly due to the commitment of other agencies in this regard. The mechanisms by which environmental agents induce cancer may be similar for all etiological agents. Radiation represents a research tool which is easy to use compared with chemical or biological agents. Understanding radiation carcinogenesis may be important in developing an understanding of carcinogenesis induced by other etiological agents. From the hazards viewpoint, radionuclides have been demonstrated to be a potent class of carcinogens and represents a risk to mankind that must be adequately evaluated. Preliminary results of current programs of the Carcinogenesis Program would indicate that in certain cases radiation and chemical agents

can act synergistically to induce cancer. The expertise available in the National Cancer Institute's intramural and contract programs can provide important direction in the assessment of radiation hazard. With the introduction of new materials, for example, the neutron-emitter Californium-252 as an isotope useful in bio-medical research and industrial applications, additional research is required to assess their potential hazards.

It is important that the NCI assist the Department of Transportation in assessing the possible effects of perturbation of the stratospheric ozone layers if supersonic transports are developed.

Proposed Course of Project:

It is expected that the liaison activities and the exchange of information with other agencies can be further expanded during the coming year. The major studies assessing the interaction of x-radiation and estrogenic hormones in inducing breast cancer and the interactions of alpha radiation and benzpyrene in inducing lung cancer will be concluding. In contrast the neutron-radiation and hormone studies have just been initiated. A contract to study UV-radiation induced skin carcinogenesis was awarded in February 1974. Results of these studies will not be known for at least 18 months.

Honors and Awards

Chief, Carcinogen Bioassay and Program Resources Branch

Director, Bioassay Operations Segment, Carcinogenesis, NCI

Chairman, NCI ad hoc Group on Animal Welfare

Member, Division of Cancer Cause and Prevention Interprogram Group, NCI

Member, Civil Defense Committee on Vulnerability of Food Crops and Livestock Production to Fallout Radiation

Member, Carcinogenesis Contract Program Management Group

Member, Interagency Collaborative Group on Environmental Carcinogenesis

Member, Information and Resources Advisory Group, Carcinogenesis, NCI

Chairman, Interagency Subtask Group on Carcinogen Testing

Member, National Cancer Panel Committee to Assess Cause and Prevention in Cancer Control Program

Chairman, Evaluation Group to Select Carcinogenesis Pathology Support Contracts

Chairman, Evaluation Group to Select Bioassay Prime Contract

Member, Workshop on Protocol for Carcinogen Testing

Publications

Mohr, U., Schuller, H., Reznik, G., Althoff, J., and Page, N.: Breeding of European Hamsters. J. Amer. Assoc. Lab. Anim. Sci. 23(6): 799-802, 1973

Linhart, M.S., Cooper, John A., Martin, R. L., Page, N. P., Peters, J. A.: Carcinogenesis Bioassay Data System. J. Comp. Biomed. Res. (In Press)

SUMMARY REPORT

CARCINOGEN METABOLISM AND TOXICOLOGY BRANCH

July 1, 1973 through June 30, 1974

Plans, develops, and conducts a research program including (1) identification, chemical analysis, mode of formation, and metabolism of various classes of chemical carcinogens; (2) studies on the toxicology and metabolic pathways of carcinogens in selected animal systems and in relation to man; (3) correlation of chemical and toxicological data for the selection and screening of chemical carcinogens; (4) development of biological and biochemical methods for the identification of reactive carcinogenic metabolites; and (5) metabolic activation of carcinogens by bacteria in the digestive tract.

The Carcinogen Metabolism and Toxicology Branch was established in April 1973 from the previously existing Carcinogen Screening Section of the Experimental Pathology Branch and the Analytical Chemistry Unit which had been working within the Office of the Associate Director for Carcinogenesis until the reorganization to form this Branch. The Carcinogen Screening Section was mainly involved in the development and monitoring of screening programs for the detection of carcinogenic hazards, investigating the pharmacological activities of selected chemicals, and exploring metabolic pathways of selected test chemicals.

The senior staff of the Branch is heavily involved in the scientific direction of collaborative projects, through several Segments of the Carcinogenesis Program. The Carcinogen Metabolism and Toxicology Segment, supported by staff from this Branch, coordinates contracts on the toxicology and metabolism of naturally occurring carcinogens and the program on nitrosamines. The Branch staff has initiated and monitors several contracts on development of short-term bioassay systems as potential prescreens for carciogens based on mutagenicity of bacterial and mammalian cells and on either *in vitro* or in host-mediated assays. Senior staff is also involved in the selection of chemicals for the bioassay program. The staff of the Bacterial Metabolism Unit is largely responsible for the activity of the Colon Cancer Segment.

Metabolic Studies Section - *Plans and conducts research including (1) identification, mode of formation and metabolism of various classes of chemical carcinogens; (2) studies on the toxicology and metabolic pathways of carcinogens in selected animal systems and in relation to man; and (3) correlation of chemical and toxicological data for the selection and screening of chemical carcinogens.*

During the past year this Section has continued the study on the chemotherapeutic agent 6-aminochrysene. Using ring-labeled 6-aminochrysene, it was determined that most of the compound was excreted extremely slowly after a single injection. The main metabolites which could be identified were the N-glucuronide of 6-aminochrysene and 6-acetylaminochrysene although some hydroxylated metabolites were formed. They were not identical with the reference compounds available, namely 12-hydroxy-6-acetylaminochrysene and

N-hydroxy-6-acetylaminochrysene. During the past year the Section has done much of the preliminary work necessary for a metabolic study of the environmental carcinogen 2,4-toluenediamine. This compound is of importance as a dyestuff intermediate and especially as the basis for most of the polyurethan foams currently in use. Many possible metabolic intermediates of 2,4-toluenediamine have been synthesized. Methods for their detection and quantitation have been worked out and some *in vitro* studies involving enzyme and cell systems have begun. Confirmation of the metabolic studies awaits the arrival of the carbon-14 labeled material from a commercial supplier.

A study was done during the past year of the comparative metabolism of 2-acetylaminofluorene in a very resistant and in a sensitive species of mouse, namely the X-GF mouse which does not develop tumors even when fed high levels of N-2-fluorenylacetamide for their lifetime (provided by Dr. A. Goldfeder of Columbia University), and the sensitive NIH-Swiss mouse. A metabolic study showed that the excretion of various metabolites was very similar in both strains. Even the resistant X-GF mice excreted the important metabolite N-hydroxy-2-fluorenylacetamide in amounts comparable to those excreted by the sensitive mouse. To resolve the question of why one strain of mice is more resistant than the other, further studies on crucial enzymes will be necessary.

In view of the Section's experience in separating the water soluble metabolites of the reference compound acetanilide, similar metabolites from N-2-fluorenylacetamide have been investigated. New column systems are being used to separate these compounds. It is hoped that these techniques will lead to positive identification of the water-soluble metabolites of the carcinogen.

Liver cell cultures are being used as a means for studying the carcinogenic process *in vitro*. During previous years a method was developed for culturing rat liver parenchymal cells which could be transformed by various chemical carcinogens. During the past year studies have been initiated on the biochemical characteristics of some of these cells, especially on the aryl hydrocarbon hydroxylase (AHH) levels and the levels of this enzyme following repeated passages or after freezing. Freezing of the cells did not affect the AHH levels, making it possible to stock low passage cells for further biochemical determinations.

Studies on the chemical induction of intestinal tumors and some of the factors affecting tumor response have continued. The two colon tumor lines which were first transplanted at the beginning of last year have been continued; both lines have the histological characteristics of mucinous adenocarcinomas. The tumors now grow quite vigorously and have been provided to several other investigators. In collaboration with the Biology Branch some immunological studies on inhibition of the growth of this type of tumor have begun.

Previously investigators in this laboratory and many others have found that azoxymethane is an excellent model compound for inducing small intestinal and colon cancers in rats and mice. In collaboration with Dr. R. Moss of Rutgers University, it was found that a change in chemical structure led to a marked difference in the organs affected. Octylazoxymethane was much less toxic than azoxymethane in mice. In addition it did not induce any intestinal tumors but led to bladder and lung tumors after a single dose.

In collaboration with Dr. Arthur Cohen of the Keene Clinic, Keene, New Hampshire, a study was recently completed on facile induction by dibutylnitrosamine of bladder tumors in rabbits, a species which is readily cystoscoped so that the course of the tumor in the bladder can be followed over a period of many months. This project has been completed and a manuscript submitted for publication.

Analytical Chemistry Unit: The purpose of the Analytical Chemistry Unit as presently constituted is threefold: (1) To collaborate with other members of the Carcinogenesis staff in matters requiring chemical expertise, especially on problems on the identification of non-polymeric organic molecules; (2) to participate in the monitoring of contract activities involving chemistry; and (3) to conduct independent research on the chemistry of N-nitroso compounds, and to serve as a focal point in the Carcinogenesis Program for the exchange of information concerning this class of carcinogens.

The well-equipped, high resolution mass spectrometry laboratory, now in full operation, provides the cornerstone of the Unit's intramural collaborative efforts with services and collaborative assistance available to the research staff of the Carcinogen Metabolism and Toxicology Branch as well as to the workers of the entire Carcinogenesis Program. Structural analysis is feasible on nonpolymeric organic compounds in the solid, liquid or gaseous form. A gas chromatograph is interphased directly with the mass spectrometer for analysis of those mixtures or their derivatives that can be fractionated by this type of chromatography. A project on the gas chromatography-mass spectrometry of sugar trimethylsilyl ethers has been completed, and a wide variety of aromatic amines, polynuclear hydrocarbons, bile acids, N-nitroso compounds, and their metabolites have been studied in collaboration with scientists outside the Unit. The mass spectrometer laboratory is involved in the following activities:

The gas chromatography-mass spectrometry system has been put to very valuable use in supporting synthetic and mechanistic studies on dialkylnitrosamines, an area of major interest for the investigators in the Analytical Chemistry Unit.

A number of mass spectra were run on various synthetic 6-aminochrysene derivatives with the aim of facilitating the structural elucidation of metabolic products from the parent compound.

Mass spectral measurements assisted in the structure determination of synthetic intermediates during the preparation of various 2,4-diaminotoluene derivatives for the purpose of metabolic studies.

In informal collaboration with Dr. J. Selkirk of the Chemistry Branch, mass spectrometric analysis is being used to determine the structure of benzo(a)-pyrene metabolites in liquid chromatographic eluates.

In informal collaboration with Dr. L. DeLuca of the Lung Cancer Branch, plans are made to develop an analytical method for structure determination of biologically important vitamin A conjugates (retinyl-phosphate-mannose type compounds).

In informal collaboration with Dr. Thomas Orme of the Viral Biology Branch, Viral Oncology Program, a gas chromatographic method is being developed for analysis of tumor cell wall carbohydrates. A manuscript describing initial results for analysis of hexosamines has been submitted for publication.

Data are being collected on the mass spectra of nitrosamines in particular. Synthesis of several deuterium labeled dialkylnitrosamines is planned to elucidate molecular fragmentation processes taking place in the mass spectrometer.

In informal collaboration with Dr. H. Schichi of NEI and with Dr. F. Harosi of NINDS, structural studies using mass spectrometry are being done on the Schiff bases of retinal with O-methylhydroxylamine and with N-α-acetyl-L-lysine methyl ester as possible chemical models in studying the mode of action of rhodopsin.

Some mass spectra are determined, as a time available basis, for investigators of Dr. John Driscoll's group (Drug Development Branch, Division of Cancer Treatment) in return for use of other needed equipment, such as infrared and nuclear magnetic resonance instruments and a polarimeter.

In addition, the Unit's research activities are aimed at plugging the important gap in the understanding of the possible role of N-nitroso compounds in environmental carcinogenesis, which resides in the fact that their simple organic chemical properties have never been fully characterized. These findings, as reported in individual project reports, may provide important background and leads for developing analytical methods for elucidating the environmental distribution of these compounds and for understanding the biochemical and biological consequences of exposure to them.

In addition to these original research activities, Unit personnel are involved with an NCI committee for setting safety guidelines in handling of chemical carcinogens, and are members of two segment advisory groups, including the Carcinogen Metabolism and Toxicology Segment, and the Information and Resources Segment, and its Chemical Selection Committee. Besides acting as project officer on a variety of contracts, Unit personnel have been involved in developing, evaluating, and reviewing proposals for contracts in various areas related to N-nitrosamines. Excellent communications have been developed between members of this Unit and other investigators working in the field of nitrosamines.

Bacterial Metabolism Unit: This Unit, whose functions were previously assigned to the recently abolished Epidemiologic Pathology Unit in the Office of the Associate Director for Carcinogenesis, is located at the NCI's Frederick Cancer Research Center (FCRC). Although scientific direction of the Colon Cancer Segment occupies most of the Unit's time, a research program in collaboration with scientists at the FCRC has been developed that gives the Unit a chance for direct involvement in the laboratory investigations that lie at the heart of the Colon Cancer Segment.

A study of the metabolism of bile acids by anaerobic intestinal microorganisms is underway. A semi-defined medium for the growth of several species of anaerobic bacteria was developed for use in this program. Yeast extract is

the only undefined ingredient and, as yet, no chemically defined substitute has been identified. Methods have been devised for extracting bile acids and their derivatives from culture media. The host mediated assay of Legator and Malling and the *in vitro* mutagenesis technique of Ames have been investigated as bioassay methods for screening bacterial cultures. Although the response obtained with individual mice was quite variable in the host mediated assay with a sufficient number of animals suitable for statistical analyses, the test should prove satisfactory. Several strains of mice, routes of injection and time intervals for various stages were studied. All strains of mice behaved in a similar manner. Thirty-sex acid sterols were tested for a co-mutagenic effect with N-methyl-N'-nitro-N-nitrosoguanidine. No enhanced mutagenesis was observed with any sterol.

Serial No. NCI-4500
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mode of Action of Chemical Carcinogens -- The Mechanisms of the Appearance of α-Fetoproteins (AFP) in the Serum of Rats Given Chemical Carcinogens

Previous Serial Number: Same

Principal Investigator: James M. Sontag, Ph.D.

Other Investigators: Elizabeth K. Weisburger, Ph.D. and Jerrold M. Ward, D.V.M., Ph.D.

Cooperating Units: None

Man Years:

Total	0.3
Professional	0.1
Other	0.2

Project Description

Objectives:

A fetal globulin, α-fetoprotein (AFP) has been demonstrated to be present in the serum of some patients with hepatocellular carcinoma and a few other lesions such as teratomas. Depending on geographic location, the percentage of AFP-positive patients varies from 30% in some areas like the United States or Great Britain, to 80% in Africa. The presence of AFP is being developed as a diagnostic tool for hepatocellular carcinomas. A number of questions on the mechanism of formation and of the derepression of this fetal protein are of interest in relation to the etiology of hepatocellular carcinoma in man. Do all hepatocarcinogens lead to tumors elaborating AFP? Does age play a role? Does the morphology and differentiation of the tumor affect production? Some of these basic questions were investigated in rats.

Methods Employed:

Utilizing amniotic fluid of inbred female Fischer strain rats at 16 to 20 days of gestation as antigen, antiserum was raised in rabbits. The antiserum was purified by specific techniques to secure an AFP-specific antiserum.

Double diffusion (Ouchterlony) techniques were utilized to assess the presence of AFP in serum of treated and control rats. In some cases confirmatory experiments were performed by electrophoresis and immunoelectrophoresis on cellulose acetate strips or on agarose coated slides.

In addition, radioimmune assays of selected serum samples have been performed by Dr. Sell, University of California, San Diego.

Major Findings:

Presence of α-fetoprotein (AFP) in serum of rats fed the hepatotoxin or hepatocarcinogen aflatoxin. Female Fischer rats were exposed to 2 ppm of aflatoxin B_1 from the time of conception to weaning of their offspring. The offspring received the same diets after weaning until their death. AFP levels have been followed in the sera of rats fed these diets. With the Ouchterlony technique it is practically impossible to detect AFP in the sera of rats fed low levels of aflatoxin B_1. However, with the radioimmune assay method of Dr. Sell, AFP levels in the serum samples were increased.

Significance to Biomedical Research and the Program of the Institute:

The current test series were inspired by the differences in the presence of a diagnostic antigen, α-fetoprotein (AFP) in the serum of human patients with liver cancer. The etiology of liver cancer in different parts of the world is unknown but presumably rests on a variety of inciting factors and chemical carcinogens. Model studies to pinpoint specific carcinogenic agents which would give rise to AFP and others which would not, might give clues to etiologic factors operating in man in various regions. At the same time an understanding of the factors controlling the release of a fetal antigen from normal or cancer cells might further our understanding of the mechanisms of carcinogenesis and thus eventually yield information which can be used to control and perhaps prevent cancer development. Studies of the effect of immunocompetence in the carcinogenic process likewise bear on these same problems and their resolution would certainly augment means of controlling the progress of the disease.

Proposed Course of Project:

For two reasons this project will be terminated some time during the next Fiscal Year. One is the lack of personnel in the Branch to carry out such experiments, the other is that considerable effort in this area is being done under a contract with Dr. T. Borsos of the Biology Branch. Therefore, when studies now underway are completed the project will be terminated.

Publications

Kroes, R., Williams, G. M., and Weisburger, J. H.: On the precocious induction of α-fetoprotein by several hepatocarcinogens, including aflatoxin B_1, in rats. Proceedings of Primosten, Yugoslavia meeting (in press).

Serial No. NCI-4580
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Role of Lipotropes in Carcinogenesis

Previous Serial Number: None

Principal Investigator: Lionel A. Poirier, Ph.D.

Other Investigators: Jerrold M. Ward, D.V.M.,Ph.D. and Timothy Benjamin, B.S.

Cooperating Units: Dr. E. L. R. Stokstad, University of California, Berkeley
Dr. Russell Madison, Microbiological Associates
Dr. D. M. Matthews, Westminster Square Hospital, London

Man Years:

Total	:	0.9
Professional	:	0.3
Other	:	0.6

Project Description

Objectives:

There is evidence that certain dietary deficiencies may lead to necrosis and fat deposition in the liver. Such morphological changes may be a prelude to eventual development of a neoplastic condition. Therefore, investigations are pursued on the extent to which the lipotropes, choline and methionine, inhibit, and vitamin B_{12} accelerate chemical carcinogenesis. The effects of these substances on chemical carcinogenesis by a variety of carcinogens are to be investigated. In addition, the mutual interactions of the lipotropes and the chemical carcinogens are to be determined.

Methods Employed:

The toxicity and the carcinogenic activity of 2-acetylaminofluorene (150 ppm in the diet), N,N-dimethyl-4-aminoazobenzene (700 ppm in the diet), aflatoxin (3 ppm in the diet), and diethylnitrosamine (40 ppm in the drinking water) were studied in rats fed a vitamin B_{12}-deficient diet and the same diet supplemented with 100 micrograms per kg of vitamin B_{12}. *S*-Adenosylmethionine was determined by thin-layer and column chromatography. Folate determinations

were made using *L. casei*, *P. cerevisiae* and *S. faecalis*, following column chromatographic separations of liver folates. Vitamin B_{12} derivatives were studied by thin-layer chromatography and microbiological assays.

Major Findings:

In studies done in collaboration with Dr. Russell Madison, the carcinogenic activity of 2-acetylaminofluorene, N,N-dimethyl-4-aminoazobenzene, and diethylnitrosamine towards rat liver was found to be greater in rats receiving a vitamin B_{12}-deficient diet supplemented with vitamin B_{12} than in rats receiving the B_{12}-deficient diet alone. The carcinogenic action of aflatoxin was not significantly altered by vitamin B_{12}. All compounds appeared to be more toxic to the livers of vitamin B_{12}-supplemented animals than to the livers of the corresponding animals receiving no B_{12} supplement. Further, ethionine toxicity was significantly greater in rats receiving vitamin B_{12} than in animals remaining on the vitamin B_{12}-deficient diet. Collaborative studies done with Dr. E. L. R. Stokstad and with Dr. D. M. Matthews indicated that chronic diethylnitrosamine administration led to a series of significant changes in the metabolism of lipotropes. Specific changes were: decreases in hepatic folate content, especially in the pentaglutamate fraction, to a 25% decrease in the hepatic levels of *S*-adenosylmethionine and in the levels of 5-methyltetrahydrofolate:homocysteine methyltransferase. A slight increase in 5,10-methylene tetrahydrofolate reductase activity and in methylcobalamine was also noted in the livers of the diethylnitrosamine-treated rats. The total vitamin B_{12} content of rat liver appeared to be unaffected by the chronic administration of diethylnitrosamine. These results are consistent with the hypothesis that the alterations in folate metabolism are a consequence of the methyl deficiency produced by diethylnitrosamine. Other findings include the observation that the histidase and urocanase levels of a hepatoma may be induced by either ethionine or by a combination of vitamin B_{12} and methionine and choline; none of the lipotropes was effective singly. Experiments are also currently underway attempting to establish a more rapid determination of *S*-adenosylmethionine in tissues.

Significance to Biomedical Research and the Program of the Institute:

Although certain dietary factors influence greatly the course of chemical carcinogenesis, there is a dearth of detailed information on the effects of the lipotropes choline and methionine and on the effects of certain vitamins. The experiments under this project aim to delineate the exact roles of these dietary factors in hope of eventually preventing or inhibiting human cancer.

Proposed Course of Project:

The effect of vitamin B_{12} on chemical carcinogenesis will be investigated in detail with one or more of the hepatocarcinogens. The effect of vitamin B_{12} deficiency on the metabolism of 2-acetylaminofluorene and the toxicity and carcinogenicity of ethionine will be determined. The extension of the vitamin B_{12} effect on carcinogenesis in other target organs will be investigated. The effects of various carcinogens on the levels of

S-adenosylmethionine in target tissues are to be determined. The results from these experiments, as well as those from collaborating laboratories, should provide a critical appraisal of the role of lipotropes in chemical carcinogenesis.

Honors and Awards

Lionel A. Poirier:

Invited Participant: Panel on Nutrition, NAS-FDA Conference on Carcinogenesis Testing, May 23-25, 1973.

Invited Lecturer: One-Carbon Compounds in Carcinogenesis. Department of Pharmacology, University of Kentucky, Lexington, September 21, 1973.

Consultant to Working Group on preparation of report "The Testing of Chemicals for Carcinogenicity, Mutagenicity and Teratogenicity." Food and Drug Directorate, Health and Welfare of Canada.

Publications

Petri, W. A., Jr., Poirier, L. A., and Morris, H. P.: A lipotrope-dependent increase of histidase and urocanase in the livers of choline-deficient rats and in the Reuber H-35 transplanted hepatoma. Biochim. Biophys. Acta 321: 681-684, 1973.

Poirier, L. A.: Folate deficiency in rats bearing the Walker Tumor 256 and the Novikoff hepatoma. Cancer Res. 33: 2109-2123, 1973.

Serial No. NCI-4617
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Carcinogen Screening Operations

Previous Serial Number: Same

Principal Investigator: Elizabeth K. Weisburger, Ph.D.

Other Investigators: Lionel A. Poirier, Ph.D., James M. Sontag, Ph.D., Jerrold M. Ward, D.V.M.,Ph.D., Timothy Benjamin, B.S. and Carolyn A. Brown, M.S.

Cooperating Units: Dr. H. Ruelius, Wyeth Laboratories, Inc.
Dr. H. Rosenkranz, Columbia University
Dr. V. Simmon, Stanford Research Institute
Dr. J. Carter, A. D. Little, Inc.
Dr. M. Shimkin, University of California, La Jolla
Dr. B. Ulland, FCRC

Man Years:

Total	:	2.0
Professional	:	1.1
Other	:	0.9

Project Description

Objectives:

Responsibilities are assumed on the design, performance and evaluation of studies in either contract or inhouse laboratories on the chronic effect and carcinogenicity of chemicals or of mixtures of chemicals. The aim of these investigations is gather information on hazards involved in handling certain chemicals or drugs, and also on the mechanisms underlying joint effects in mixtures. Of particular interest are such mixtures which tend to give increased or decreased overall carcinogenic effect. Of concern also is the methodology of carcinogen screening, with emphasis on improvement in sensitivity, speed and economy. Short-term assays such as those based on mutagenesis are under development, taking into account the need to secure biochemical activation of most environmental agents. Guidelines for assessment of the carcinogenic potential of chemicals are developed, by consideration of their chemical structures, metabolic pathways and mutagenic activity.

Methods Employed:

Attempts are made to secure information on chemicals representing the greatest hazard to the largest number of people. Chemicals and drugs are rated as to priority on this basis as well as other criteria such as relationship to known carcinogens, epidemiologic observations, indications of specific toxicity and related factors. Standard protocols as well as newly designed protocols attempting to increase the sensitivity and speed of such tests are utilized. The carcinogenic and mutagenic activities of various compounds are determined in appropriate systems, either directly or after metabolic activation. Exploratory meetings with relevant national and international organizations in industry, in government, and in university environments are setting the stage for a concerted developmental effort in this area.

Major Findings:

In the current year several of the long term experiments in contract laboratories are almost completed even though statistical evaluation is yet to be done. A number of chemicals which are of environmental importance have been found carcinogenic in mice or rats.

Pesticides. During the past year we have definite evidence that several simple halogenated compounds which are employed as fumigants for soils, grains and some other types of food and as gasoline additives are carcinogenic in both rats and mice.

Industrial chemicals. Indications from a more rapid bioassay system using the strain A mouse are that some of the simple halogenated compounds, some of which are industrial intermediates, cause an increase in pulmonary adenomas. These results have been checked and are now ready for publication.

Natural products. Studies on fungi isolated from Asian foodstuffs are continuing. In addition, the possible carcinogenicity of fractions from plant materials used in Curacao and certain sections of South Carolina regions with high esophageal cancer incidence are being checked.

Auramine carcinogenicity and metabolism. The major question of this investigation is to determine whether the reactive moiety of auramine is the methyleneimine bridge between the two benzene rings or the dimethylamino group at either end of the molecule. Both have the potential to react as reactive electrophiles. Auramine and a series of structurally similar analogs are currently under subacute toxicity studies at FCRC for the carcinogenicity tests to be undertaken shortly. The analogs include: Michler's ketone, Michler's hydrol and *p,p'*-dimethoxybenzophenoneimine. These tests should determine the relative importance of the methyleneimine bridge and the dimethylamino group to auramine carcinogenicity. Further, the metabolism of auramine in rats and mice has been investigated. Michler's ketone has been identified as a major urinary metabolite of this compound in rats. In direct tests with a series of amino acids, auramine was found to react with the α-amino group of lysine as well

as with histidine. Mass spectroscopy indicates that the auramine-lysine adduct appeared to be an imine containing the amino group of lysine and the benzylic carbon of auramine.

Anethole and estragole carcinogenicity and metabolism. These compounds are monomethoxy analogs of the safrole series of carcinogens. It is expected that they may be similarly metabolized to benzylic alcohols. Subacute toxicity studies have indicated that both of these compounds are toxic to the livers of mice. Long term feeding studies of both compounds are currently underway.

Mutagenic and carcinogenic activities of the low molecular weight alkyl halides. The carcinogenicity of several alkyl halides were tested by lung adenoma formation in strain A mice; these studies were done in collaboration with Drs. G. Stoner and M. Shimkin. The following compounds were found to significantly increase the number of lung adenomas per mouse: methyl iodide, n-propyl iodide, i-propyl iodide, s-butyl chloride, t-butyl chloride, i-butyl bromide, s-butyl bromide, t-butyl bromide, n-butyl iodide and s-butyl iodide. The following compounds displayed no significant carcinogenic activity: ethyl bromide, ethyl iodide, n-butyl chloride, n-butyl bromide, benzyl chloride, 1-chloromethylnaphthalene. The results indicate that alkyl halides as a class seem to be weak carcinogens. Within this class, however, the normal alkyl bromides and iodides are both toxic and carcinogenic. The normal alkyl chlorides appear to be quite inert. Thus the carcinogenic activities of the n-alkyl halides parallel their chemical reactivities. The fact that all 3 t-butyl halides were active suggests that the leaving group does not play such a significant role in highly branched structures as it does in normal structures. The apparent absence of carcinogenic activity by the ethyl and the aromatic halides suggests that these may be detoxified to a greater extent than the other structures studied. In collaboration with Dr. H. Ruelius, the activities of several alkyl halides were also investigated in a repair deficient strain of *E. coli*. Compounds possessing no significant activity in inhibiting the growth of repair deficient *E. coli* included: n-butyl chloride, n-butyl bromide, n-butyl iodide, s-butyl bromide, t-butyl chloride, t-butyl bromide. The compounds found active included i-butyl bromide, s-butyl iodide, t-butyl iodide and benzyl chloride. These results indicate that Pol A (-) *E. coli* seems relatively insensitive to the toxic effects of the alkyl halides; it appears to be sensitive only to the more reactive compounds.

Mutagenicity prescreens for chemical carcinogens. This is basically a contract program to determine the extent of correlation between chemical carcinogenesis and chemical mutagenesis. Eighty-five compounds including procarcinogens, noncarcinogens, ultimate carcinogens, and mutagens are being tested for their mutagenic activity. The spectrum of mutagenic activity includes DNA repair, gene mutations in mouse lymphoma cells, and microbial gene mutations. The latter are to be measured in yeast D-3, fine strains of histidine requiring Salmonella and repair deficient *E. coli*.

All strains selected are to be tested *in vitro* and *in vitro* with metabolic activation using rat liver post-mitochondrial supernatant for their sensitivity towards each of the 85 compounds. Gene mutations in selected strains are to be observed *in vivo* with all compounds. Currently 45 compounds have been investigated for microbial gene mutation. Six compounds have been studied for their activity towards DNA repair and mammalian gene mutations. The results indicate the following: 1) All direct-acting carcinogens tested to date appear to be direct-acting mutagens. 2) Most of the procarcinogens can be detected with an appropriate genetic marker in the presence of metabolic activation. Some exceptions appear to exist: these include drugs requiring demethylation, thioacetamide and safrole.

Significance to Biomedical Research and the Program of the Institute:

Many factors in the environment have not yet been evaluated for chronic toxicity and possible carcinogenicity. It is important to secure such information after deliberate and judicious setting of priorities. Cancer in man has been observed in the past as a result of exposure to chemicals subsequently demonstrated to be carcinogenic. The aim of this program is to forestall such unintentional exposures of man which would result in neoplastic disease in the future.

Proposed Course of Project:

Continuing efforts are required 1) to assess relative priorities of materials to be tested, 2) to undertake studies on variations in the protocols to simplify or to develop novel test techniques which are more sensitive, more specific and more economic, and 3) to apply such tests to identify carcinogenic hazards in the environment.

The studies on auramine and other potential benzylic carcinogens will continue with new examples being investigated. Studies on alkyl halides will be extended to determine the effects of anchimeric assistance and physiologically important leaving groups on the response in the strain A mouse lung adenoma test. Mutagenicity-carcinogenicity studies and correlations will also continue as new substances enter the environment to provide estimates of the potential carcinogenicity of these materials, explore the activity of new structures, and possibly afford a means to screen human populations exposed to chemical carcinogens.

Honors and Awards

Lionel A. Poirier:

Invited Lecturer: Prescreens for Chemical Carcinogens. Gordon Research Conference on Toxicology and Drug Safety Evaluation, Kimball Union Academy, Meriden, New Hampshire, August 3, 1973.

Invited Lecturer: Dyes in Carcinogenesis and Mutagenesis. Food and Drug Administration, Washington, D. C., October 11, 1973.

Invited Participant: Mutagenesis Panel, NAS-FDA Conference on Carcinogenesis Testing, May 23-25, 1973.

Member, Carcinogen Metabolism and Toxicology Segment

Member, Information and Resources Segment

Member, United States Interagency Panel on Environmental Mutagens

Chairman, Subgroup on Mutagenesis of Chemical Carcinogens, CMT

Elizabeth K. Weisburger:

Invited Lecturer: Inhibitory Effects in Chemical Carcinogenesis. Laboratory of Chemical Pharmacology, National Heart and Lung Institute, Bethesda, Md., December 10, 1973.

Invited Lecturer: Safety Measures in a Carcinogenesis Bioassay Program. NAS-NRC Meeting, Chemical Safety Resources in the Washington Section, Washington, D. C., May 22, 1973.

Invited Lecturer: Inhibition of Chemical Carcinogenesis. Department of Pharmacology, University of Kentucky, Lexington, February 28, 1974.

Invited Lecturer: Methods for Determining Carcinogenicity. Food and Drug Administration, Washington, D. C., May 2, 1974.

Invited Lecturer: A Critical Evaluation of the Methods Used for Determining Carcinogenicity. Third Annual Meeting of the American College of Clinical Pharmacology, Atlantic City, New Jersey, May 4, 1974.

Invited Lecturer: Identification of Chemical Carcinogens. Symposium of American Society of Biological Chemists, Minneapolis, Minnesota, June 5, 1974.

Assistant Editor-in-Chief, *Journal of the National Cancer Institute*, 1971--

Editorial Board, *Excerpta Medica (Cancer)*

Board of Trustees, Lebanon Valley College, 1970-76

Consultant, Surgical Drugs Advisory Board, Food and Drug Administration

Member, Editorial Advisory Committee of the American Association for Cancer Research for the journal, *Cancer Research*

Member, Carcinogenesis Contract Program Management Group

Director, Carcinogen Metabolism and Toxicology Segment

Member, Bioassay Segment Advisory Group

Serial No. NCI-4617

Chairman, Chemical Selection Committee

Participant, National Cancer Plan

Publications

Baker, J. R., Smith, E. R., Yoon, Y. H., Wade, G. G., Rosenkranz, H., Schmahl, B., Weisburger, J. H., and Weisburger, E. K.: Effects of 2-amino-anthraquinone on the blood and kidneys of intact and castrated rats. Toxicol. Appl. Pharmacol. (in press).

Olson, W. A., Habermann, R. T., Weisburger, E. K., Ward, J. M., and Weisburger, J. H.: Induction of stomach cancer in rats and mice with halogenated aliphatic fumigants. J. Nat. Cancer Inst. 51: 1993-1995, 1973.

Prejean, J. D., Peckham, J. C., Casey, A. E., Griswold, D. P., Weisburger, E. K., and Weisburger, J. H.: Spontaneous tumors in Sprague-Dawley rats and Swiss mice. Cancer Res. 33: 2768-2773, 1973.

Russfield, A. B., Homburger, F., Boger, E., Van Dongen, C. G., Weisburger, E. K., and Weisburger, J. H.: The carcinogenic effects of 4,4'-methylene-bis-(2-chloroaniline) in mice and rats. Toxicol. Appl. Pharmacol. (in press).

Stoltz, D., Poirier, L. A., Irving, C. C., Stich, H. F., Weisburger, J. H., and Grice, H. C.: Evaluation of short-term tests for carcinogenicity. Toxicol. Appl. Pharmacol. (in press).

Stoner, G. D., Shimkin, M. B., Kniazeff, A. J., Weisburger, J. H., Weisburger, E. K., and Gori, G. B.: Test for carcinogenicity of food additives and chemotherapeutic agents by the pulmonary tumor response in strain A mice. Cancer Res. 33: 3069-3085, 1973.

Ulland, B. M., Weisburger, J. H., Yamamoto, R. S., and Weisburger, E. K.: Antioxidants and carcinogenesis: Butylated hydroxytoluene, but not diphenyl-*p*-phenylenediamine, inhibits cancer induction by N-2-fluorenylacetamide and by N-hydroxy-N-2-fluorenylacetamide in rats. Food Cosmet. Toxicol. 11: 199-207, 1973.

Serial No. NCI-4618
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mode of Action of Chemical Carcinogens -- Endogenous and Exogenous Factors in Chemical Carcinogenesis

Previous Serial Number: Same

Principal Investigator: Elizabeth K. Weisburger, Ph.D.

Other Investigators: Jerrold M. Ward, D.V.M.,Ph.D. and Lionel A. Poirier, Ph.D.

Cooperating Units: Dr. John H. Weisburger, American Health Foundation
Dr. Russell M. Madison, Microbiological Associates
Dr. J. D. Prejean, Southern Research Institute
Dr. Daniel P. Griswold, Southern Research Institute
Dr. Marcus M. Mason, Mason Research Institute
Dr. Arthur Cohen, Keene Clinic
Dr. Robert Moss, Rutgers University

Man Years:

Total	: 3.5
Professional	: 1.4
Other	: 2.1

Project Description

Objectives:

Investigations to discover the intimate factors involved in the initiation and development of neoplasia are explored by means of animal experiments in various species. The studies utilized a number of chemical carcinogens administered to animals under varying conditions of diet, endocrine situation or immunological status so as to gain insight into controlling mechanisms in the initiation and development of chemically induced tumors. Further efforts deal with the development of systems which lead to the prevention of the processes of chemical carcinogenesis and a study of the mechanisms related thereto.

Methods Employed:

Chemical carcinogens or unknown agents to be evaluated for carcinogenicity are administered by a number of routes to several strains of rats or mice on specific dietary regimens. Additionally, certain chemicals with specifically tailored structures or properties are given together with chemical carcinogens to determine whether tumor induction can be delayed or inhibited. Host factors such as the endocrine or immunologic status are being investigated in relation to the effectiveness of tumor formation. After judiciously selected treatment and observation periods, autopsies of the experimental animals are performed. Upon histologic processing of tissues, the results in experimental systems are carefully evaluated in relation to control groups.

Major Findings:

1. Inhibition of liver tumors from diethylnitrosamine. A study by Dr. Maling and associates of the National Heart and Lung Institute showed that dibenamine protected rats against the acute hepatotoxic effects of carbon tetrachloride, thioacetamide and dimethylnitrosamine. It was decided to determine whether this protective action still held over the extended period necessary to induce liver tumors with a dialkylnitrosamine. Male Fischer rats were fed diethylnitrosamine in the drinking water for a period of 10 weeks. Groups of these rats were also injected 3 times weekly with either 25 mg/kg or 12.5 mg/kg of dibenamine for a 10-week period. After a total of 25 weeks all the rats were killed, and a thorough examination was made to determine tumor incidence and the sites affected. As expected, the rats on diethylnitrosamine alone had many liver tumors and several of these metastasized to the lungs. In addition these rats also had numerous papillomas or carcinomas of the oral cavity, pharynx or the esophagus. The conclusion was that dibenamine inhibited the severity and decreased the incidence of liver tumors. However, there was no effect whatsoever on the incidence or severity of the tumors of the oral cavity and esophagus.

2. Induction of intestinal tumors with butylnitrosourea in mice. Several Japanese investigators reported that leukemia could be induced in mice by ingestion or injection of butylnitrosourea. In our laboratory we found that when butylnitrosourea was fed to C57Bl mice in the drinking water, thymic lymphomas were found in 50% of all mice in 3 to 6 months. In addition, a smaller number of animals developed colon and/or small intestinal tumors. The effect on intestinal tumors was investigated further by injecting C57Bl mice once with butylnitrosourea. By 40 weeks 30% of these mice developed intestinal tumors but very few leukemias. The experiment is still in progress.

3. Effects of benzene in newborn mice. There have been many case reports in the clinical literature ascribing leukemia or disorders of the hematopoietic system in man to occupational exposure to benzene. No experimental animal model has been found which simulates this effect. We therefore attempted a study in newborn C57Bl mice which should be more sensitive to any carcinogenic effect; 80 male mice were injected with benzene dissolved in

corn oil once weekly for a one year period and then were held until two years of age. Benzene did not appear to be carcinogenic for there were no differences in tumor incidence among the treated, vehicle control, and the untreated control mice. The only deleterious effect was that benzene treatment seemed to cause earlier appearance of amyloidosis than in the controls.

4. Carcinogenesis study with nitrogen mustard and phorbol esters. Nitrogen mustard has been used for treatment of certain skin disorders, especially psoriasis in patients. To determine whether there was any risk from this therapy, BALB/C female mice were painted twice weekly for 18 weeks with nitrogen mustard. A promoting agent, a phorbol ester, derived from croton oil was also applied twice weekly for 26 weeks. Observation of the mice is continuing. There was a slight increase of papilloma formation with mice receiving both treatments but no carcinomas were seen. No skin tumors were observed after treating the skin with nitrogen mustard alone. The conclusion is that nitrogen mustard is a weakly active initiator but not a complete carcinogen for the skin of mice.

5. Induction of tumors with dibutylnitrosamine. As part of a study carried out with Dr. Arthur Cohen, rabbits were injected subcutaneously with dibutylnitrosamine for periods up to 18 months. Cystoscopy of the bladders revealed the development of suspicious lesions and finally tumors from 15 to 24 months after the first injection. At autopsy histopathological examination showed that the bladders contained foci of hyperplasia, papillomas, carcinomas and hemangiomas. In addition, hyperplastic and neoplastic lesions in the lungs and kidneys were also induced. The rabbit may serve as a model for the study of development of bladder tumors since cystoscopic review is quite feasible.

6. Testing black pepper for possible carcinogenic activity in mice. Many food additives and spices to which most humans are exposed daily have not been tested for possible carcinogenic activity, even though the causes of many human cancers are not known. A commonly used spice, ground black pepper, was fed to NIH-Swiss mice at the maximally tolerated dose or 2% of the diet. By one year only one of the 40 mice had died of any tumor and the controls had remained normal. The experiment is still in progress.

7. Octylazoxymethane in mice. Azoxymethane has been employed to induce colon tumors in mice and rats. In order to determine the effect of replacing one of the methyls by an octyl group, octylazoxymethane was synthesized by Dr. Moss of Rutgers University. The compound was tested in NIH-Swiss male mice which were injected with octylazoxymethane weekly for 14 weeks. It was noted that this compound was much less toxic than azoxymethane and much higher doses were required to induce weight loss or any other toxic symptoms. However, by 40 weeks 50% of the mice had malignant bladder tumors and multiple lung tumors. In contrast azoxymethane induces mostly colon tumors and very few lung tumors. Thus a simple structural change was responsible for a decidely different organotropic effect.

8. Effect of aflatoxin on fetus. Aflatoxin is suspected as a cause of liver cancer in some parts of the world where high humidity and poor storage conditions lead to rapid contamination of the peanut or other grain crops. In order to check whether *in utero* exposure leads to rapid liver cancer induction, female rats received 2 ppm of aflatoxin B_1 from the time of conception to weaning of the offspring. These offspring then received the same diet after weaning until death while another group of Fischer rats received 2 ppm of aflatoxin to 6 weeks of age until death. Early exposure to aflatoxin resulted in a two month decrease in survival time. As compared with rats receiving aflatoxin 6 weeks after birth, more of the rats exposed *in utero* died of hemorrhage from anaplastic liver tumors or hemangiomas than did rats exposed 6 weeks of age onward. The experiment is still continuing for some of the animals are still alive and the data need to be analyzed statistically.

Significance to Biomedical Research and the Program of the Institute:

The prevention and cure of neoplasia hinges on an understanding of the intimate factors involved in the pathogenesis of cancer. The research performed within the framework of this report aims to produce data which will permit the comprehension of the underlying mechanisms involved in chemical carcinogenesis.

Proposed Course of Project:

A number of the tests for chronic toxicity and carcinogenicity presently underway or planned to go on stream shortly will require considerable effort for several years. Additional emphasis will be placed on methods for inhibiting the carcinogenic process and on understanding the various steps leading to such inhibitions. Thus studies will include variations in endogenous factors as well as select exogenous materials.

Publications

Cohen, A. E., Weisburger, E. K., Weisburger, J. H., Ward, J. M., and Putnam, C. L.: Cystoscopy of chemically-induced bladder neoplasms in rabbits administered the carcinogen dibutylnitrosamine. Investig. Urol. (in press).

Mason, M., Baker, J. R., Yerganian, G., Weisburger, E. K., and Weisburger, J. H.: Induction of tumors of the stomach and esophagus in inbred Chinese hamsters by oral diethylnitrosamine. Proc. Soc. Exp. Biol. Med. (in press).

Weisburger, E. K., Ward, J. M., and Brown, C. A.: Dibenamine: Selective protection against diethylnitrosamine-induced hepatic carcinogenesis but not oral pharyngeal and esophageal carcinogenesis. Toxicol. Appl. Pharmacol. (in press).

Weisburger, J. H.: Chemical carcinogenesis. In Holland, J. F. and Frei, E. (Eds.): Cancer Medicine. Philadelphia, Lea and Febiger, 1973, pp. 45-90.

Weisburger, J. H., Weisburger, E. K., Madison, R. M., Wenk, M. L., and Klein, D.: Effect of acetanilide and *p*-hydroxyacetanilide on the carcinogenicity of N-2-fluorenylacetamide and of N-hydroxy-N-2-fluorenylacetamide in mice, hamsters, and female rats. J. Nat. Cancer Inst. 51: 235-240, 1973.

Yamamoto, R. S., Richardson, H. L., Weisburger, E. K., Weisburger, J. H., Benjamin, T., and Bahner, C. T.: Carcinogenicity of proposed cancer chemotherapeutic agents with stilbene-arylnitrosamine structures. J. Nat. Cancer Inst. 51: 1313-1315, 1973.

Serial No. NCI-4680
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mode of Action of Chemical Carcinogens -- Development and Application of *In Vitro* Systems Involving Epithelial Cells

Previous Serial Number: Same

Principal Investigators: Jane Idoine, B.S. and James M. Sontag, Ph.D.

Other Investigators: Robert Kroes, D.V.M., Elizabeth K. Weisburger, Ph.D., and Jerry Elliott, B.S.

Cooperating Units: Dr. Robert Huebner, Viral Carcinogenesis Branch, NCI
Dr. Aaron Freeman, Microbiological Associates
Dr. Rhim, Microbiological Associates
Dr. John H. Weisburger, American Health Foundation
Dr. Kenneth Cohen, Metabolism Branch, DCBD, NCI
Dr. George Todaro, VLL, DCCP, NCI

Man Years:

Total	1.9
Professional	0.6
Other	1.3

Project Description

Objectives:

Successful culture of epithelial-like cells derived from livers of 8 to 10 day old Fischer strain 344 rats and malignant transformation of such cultured cells by a variety of chemical carcinogens were discussed in previous reports. Since neoplasia in man and animals involves mainly epithelial cells, our principal aim in current investigations has been to further determine the potential of *in vitro* epithelial systems for use in rapid bioassay procedures for chemical carcinogens and in examining the biochemical mechanisms of carcinogenesis.

Two problems encountered in past investigations were the relatively long time required to develop cultures that were proliferating adequately to provide enough cells for biochemical investigations and the fact that some sublines of control cells underwent spontaneous malignant transformation as demonstrated

by tumor formation following injection of cells into syngeneic hosts. Unfortunately, no distinctive morphological changes were apparent in the cultured cells that induced tumors. In addition, the spontaneous transformation was not correlated with a specific time in culture nor with the number of times a line had undergone subculturing. Therefore, we have emphasized the development of methods for producing a greater number of cultures with similar characteristics so that studies can be repeated as required in more replicable systems. Investigation of pertinent biochemical properties of untreated or chemically exposed cultured cells in respect to the effects of various cultural conditions and in comparison to the characteristics of liver tissue from whole animals should help assess the value of this *in vitro* system.

Methods Employed:

Cell culture methods designed for selection of epithelial cells were employed to initiate cell cultures from livers of 8 to 10 day old Fischer rats. The method for selection of epithelial cells developed in this laboratory was modified by detaching and transferring islands of epithelial cells so that many homogenous sublines could be cultured from one primary culture. Frozen stocks of all sublines were prepared as soon as cultures were proliferating adequately, and some sublines were cultured continuously under conditions where continuing growth and proliferation were maintained. Transformation was monitored by testing cells for growth in soft agar (Sachs' method) and for ability to induce tumors after injection into animals. Biochemical procedures were applied to characterize these cells. Base levels and inducible levels of aryl hydrocarbon hydroxylase (AHH) activity were determined in homogenates of whole cells to evaluate the functional quality of the cultured cells under various conditions.

Major Findings:

1. Base levels of aryl hydrocarbon hydroxylase (AHH) activity in cultured liver cells compared to that in livers of 8 to 9 day old rats. Base levels of AHH activity were determined in 8 sublines of epithelial cells from livers of 7 and 10 day old Fischer rats. The sublines had been in culture for 2.5 to 4.5 months and had undergone 4 to 8 passages (subcultures with trypsinization). Specific AHH activity (pmoles 3-OH-benzo(a)pyrene produced from benzo(a)pyrene substrate per mg cellular protein) of 7 day old cultures, at 90 to 100% confluency, was 8.3 to 13.4 units per min. of incubation. This was comparable to the activity observed in four homogenates of liver from 8 and 9 day old Fischer rats, i.e. 7.6 to 15.7 units per min.

2. Effect of various conditions of culture on hepatic cells *in vitro*. Cells from cultures at or near confluency consistently demonstrated somewhat higher (1.5 to 1.7 times) specific AHH activity than cells of parallel cultures that were at less than 75% confluency. The higher level of enzyme activity persisted for 5 to 7 days after cultures reached confluency but decreased in older cultures (14 days) even when cells were maintained with media changes twice weekly.

The time interval between changing media on cultures and harvesting cells for assays affected the level of AHH activity. Specific activity of comparable cells was twice as high 3 to 6 hours after complete media change than that observed 16 to 24 hours after the change.

Four sublines were maintained in continuous culture for 7 to 9 months and tested at intervals for levels of AHH activity, ability to grow in soft agar, and to induce tumors after inoculation into animals. At passages 20 to 22 three of the four sublines under study demonstrated only 15 to 35% of the AHH activity observed in cells of early passages (P4 to P8), as described, while 75% of the initial activity was observed in cells of passage 22 of the fourth subline. Morphologically, all four lines appear primarily epithelial in nature as they did when first tested, although in one of the lines with sharply decreased AHH activity some spindle cells are now observed. Irradiated weanling Fischer rats inoculated with cells from the 4 sublines at passages 20 to 22 have not developed apparent tumors in the 90 days since inoculation.

3. Effect of frozen storage on cultured epithelial cells. Frozen stocks of cells prepared from cultures at the earliest possible passage levels could provide the means for experimentation in more comparable cultures than those available from lines in continuous culture. To assess the quality of cultures prepared from frozen stocks, a continuing study is underway to determine the effects of frozen storage on the biochemical characteristics of cultured epithelial cells and on their tendency toward spontaneous transformation. Portions of a pooled suspension of cells from one of the sublines described were frozen in a number of separate vials. Other parallel cultures of the same subline were used for control testing of the condition of the cells not subjected to freezing. At intervals some frozen cells were thawed, cultured, and subcultured 2 or 3 times to obtain enough cells for AHH assays, soft agar tests, and animal inoculations. Results for cultures frozen for 24 hours and for 30 days indicate that these cells have the high AHH activity observed in the cultures at the passage level from which the frozen stocks were prepared, while cells of the same line carried in continuous culture over this same period had decreased enzyme activity. Animals inoculated with cells cultured from the frozen stocks have not developed tumors in the 90 and 120 day intervals since they were injected.

4. Evidence of RNA-dependent DNA polymerase (reverse transcriptase) in cultures of chemically transformed epithelial cells. Two sublines of epithelial cells that were transformed by chemicals (aflatoxin and nitrosomethylurea), as described in earlier reports, were found to be positive for reverse transcriptase in tests employing a viral RNA template. Cultured cells from a tumor initiated by injection of the aflatoxin-treated cells were also positive. The parent line of hepatic cells from which the subline for aflatoxin treatment was prepared was negative for the reverse transcriptase as were the four sublines derived from primary cultures in the past year. The tests for the reverse transcriptase were performed through the cooperation of Dr. George Todaro's laboratory.

5. Inducibility of AHH activity in cultured epithelial cells. One of the sublines of epithelial cells that had been cultured continuously for 10 months was tested at passages 24 and 25 for inducibility of AHH activity by benzanthracene, 3-methylcholanthrene and 7,12-dimethylbenz(a)anthracene. All 3 chemicals induced significantly higher levels of AHH activity in the cultured cells.

6. Growth of hepatic cells in soft agar. As mentioned in sections 2, 3, and descriptions of methods employed, transformation was monitored by testing cells for their ability to grow in soft agar (Sachs method). Cultured cells which had been transformed chemically as well as cultured cells from a tumor initiated by injection of chemically transformed cells grew rapidly and formed large colonies in soft agar. Cultured cells which had undergone spontaneous transformation, however, grew much slower in soft agar and in some cases may have only remained viable and not proliferated. Recently initiated sublines described above which have not, as far as is known, undergone transformation either deteriorated or remained viable without proliferation in soft agar. The difficulty in detecting either growth or only continued viability in soft agar is caused by an inability to break up clumps of cells completely.

7. Production of a growth factor in cultured liver cells. It has been reported that the active agent in the *in vivo* effect of growth hormone is a protein produced by the liver. Conditioned media from one of the sublines of epithelial cells was tested and results indicated that these cells produced a heat stable non-dialyzable growth factor, which may or may not be identical with that protein (MSA). Testing of the media for the growth factor (MSA - multiplication stimulating activity) was performed in the laboratory of Dr. Kenneth Cohen.

Significance to Biomedical Research and the Program of the Institute:

An assay system which can be designed to give results more rapidly, specifically, and reliably than the customary long-term tests for carcinogenicity is highly desirable with the dual purpose of studying fundamental phenomena and the detection of harmful agents in our environment. Full exploitation of workable *in vitro* systems has not been achieved. It is our goal to develop such systems; an additional advantage of the *in vitro* systems is that eventually they can be established utilizing cells from man. Hence carcinogenesis *in vitro* with human cells avoids the question of species difference, so often raised in evaluating the significance of carcinogen bioassay systems.

Proposed Course of Project:

These epithelial cells will be carefully examined for suitability as a carcinogen screening system. The relevant morphological properties in both normal and carcinogen treated cells will be investigated thoroughly. Extensive efforts will deal with the biochemical properties of both the normal and the transformed cells in culture. Basic biochemical mechanisms of the system will be investigated.

Publications

Fish, D. C., Dobbs, J. P., and Elliott, J. M.: Effect of osmotic pressure, Na^+/K^+ ratio and medium concentration on the enzyme activity and growth of L cells in suspension culture. In Vitro 9: 108-113, 1973.

Freeman, A. E., Weisburger, E. K., Weisburger, J. H., Wolford, R. G., Mawak, J. M., and Huebner, R. J.: Transformation of cell cultures as an indication of the carcinogenic potential of chemicals. J. Nat. Cancer Inst. 51: 799-808, 1973.

Serial No. NCI-4688
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mode of Action of Chemical Carcinogens -- Investigations of Systems Leading to Cancer in the Colon and Small Intestine

Previous Serial Number: Same

Principal Investigator: Jerrold M. Ward, D.V.M., Ph.D.

Other Investigators: Richard S. Yamamoto, Sc.D., Robert C. Bast, Jr., M.D., Carolyn A. Brown, M.S., and Timothy Benjamin, B.S.

Cooperating Units: None

Man Years:

Total	:	2.1
Professional	:	0.6
Other	:	1.5

Project Description

Objectives:

In the United States the mortality rate due to colon and rectum cancer in males is second only to lung cancer, and in females second to breast cancer. In incidence, colon cancer is third after skin and lung in men, and in women, after breast and uterus. In Western Europe, the relationship is similar. However, in other countries such as Japan and Africa, colon cancer incidence is much lower, thus suggesting that environmental factors are associated with the etiology of this important disease. Because of the absence of adequate tools to induce colon and rectal cancer in animals, model studies could not be performed. However, recently several new agents were discovered by which colon cancer could be induced in animals. The goal of the studies to be described is to evaluate how select environmental factors affect the production of cancer of the colon in animals thereby gaining insight into conditions possibly affecting humans.

Methods Employed:

Rats, mice and other rodent species were treated with chemicals known to lead to colon cancer. These include 1,2-dimethylhydrazine and azoxymethane. The animals are placed on diets mimicking those utilized in various parts of the world with regard to quantity and quality of protein, fat, carbohydrates, micronutrients, and the amount of bulk material such as cellulose in the diet. After appropriate periods of time the animals are carefully autopsied and the lesions are examined microscopically.

Major Findings:

1. Dose response and the distribution of tumors in the intestines. In man colon tumors occur predominantly in the sigmoid colon in geographic areas of high incidence while in areas with a low incidence this type of tumor appears in the ascending colon. However, in rats injected with azoxymethane high doses of the carcinogen induced primary tumors of the descending colon but low doses induced more tumors in the ascending colon. In addition, high doses induced many duodenal tumors but few occurred at low doses. Diets also had some effect on the distribution of the intestinal tumors. These studies are continuing.

2. Morphology of chemically induced colon and small intestinal tumors in rats. In a study designed to follow the sequential morphological development of intestinal tumors 60 rats were injected with 1,2-dimethylhydrazine and killed at intervals from 20 to 38 weeks after the first injection, while 25 rats were allowed to die spontaneously. Three types of colon tumors were induced and were classified as polypoid, neoplasia and adenocarcinomas and mucinous adenocarcinomas. Each type developed in a specific manner. Thus the polypoid tumors always showed invasion of the stalk but rarely spread behind the submucosa. The mucinous adenocarcinomas were the most malignant types almost always arising in the epithelium over lymphoid follicles in the ascending colon and metastasizing to the abdominal cavity and lungs. All three types of tumors resemble those seen in man.

3. Transplantation of colon and small intestinal tumors and studies in immunotherapy. Chemically induced carcinomas of the small and large intestine were transplanted to weanling rats (see report of last year). These carcinomas were transplanted easily when the metastatic peritoneal tumors were used for transplantation. After several passages, the lines became more malignant. Thus after i.p. injection of 0.5 ml of tumor suspension rats died 25 days later. After s.c. injection they died after 60 to 90 days from pulmonary metastases. These tumors are being furnished to various investigators for different studies on colon tumors. In addition, in cooperation with the Biology Branch, the transplantable colon carcinoma was used to test the effect of Ribi vaccine (BCG derived). The vaccine prolonged the survival of the tumor bearing rats and prevented the growth of tumors in 50% of the rats tested. Studies on this area to determine effect of vaccine on tumor growth in colon are continuing.

4. Effect of diet in colon carcinogenesis. Rats were placed on a diet which was either deficient or adequate in vitamin B_{12} level and injected with azoxymethane. After 26 weeks the rats were killed and the number of tumors in the intestine was tabulated. Rats on the deficient diet had fewer tumors in the intestines, especially the colon, than did rats on the normal diets. However, this difference was not significant statistically. Since there was a decided weight depression in the rats on the deficient diet, the decreased effect of the carcinogen may have come from the dietary deficiency.

5. Induction of colon tumors in rats fed aflatoxin. Newberne and Rogers have recorded that aflatoxin has induced colon tumors in some rats on a diet with marginal vitamin A levels. In our studies a careful examination of the entire intestinal tract was made in all rats fed 2 ppm of aflatoxin B_1. In 44 rats on aflatoxin B_1 which died of liver tumors, 9 developed tumors of the colon. Thus, aflatoxin induced tumors not only in the liver and kidney as recorded previously but also in the colon.

Significance to Biomedical Research and the Program of the Institute:

Establishment of a colon cancer model with similarities to the human disease was necessary to study the effects of diet on colon cancer induction. Dietary factors are thought to be responsible for the occurrence of human colon cancer. Our studies suggest that under our experimental conditions, diets containing nutrients thought to be important for the high incidence of human colon cancer in some parts of the world did not increase the incidence of chemically-induced cancer. The limitations of this model are obvious.

Proposed Course of Project:

To complete all the experimental studies noted above in order to a) characterize the pathology of the experimental model, b) determine the effects of various diets on colon cancer incidence in the experimental model, c) establish the transplantable carcinomas noted above.

Honors and Awards

Jerrold M. Ward:

Invited Lecture: Experimentally-Induced Colon Cancer in Rats and Mice. Annual Meeting of the American Veterinary Medical Association, Philadelphia, Pennsylvania, July 23, 1973.

Invited Lecture: Pathology of Stomach Cancer Induced by Ethylene Dibromide and Dibromochloropropane. Meeting of the International Academy of Pathology, San Francisco, California, March 13, 1974.

Invited Lecture: Colon Cancer in Man, Dogs and Rodents. University of California, Davis, March 12, 1974.

Publications

Ward, J. M., Yamamoto, R. S., Benjamin, T., Brown, C. A., and Weisburger, J. H.: Experimental colon cancer in rats and mice. J. Amer. Vet. Med. Assoc. (in press).

Ward, J. M., Yamamoto, R. S., Benjamin, T., and Weisburger, J. H.: Transplantation of chemically-induced neoplasms of the colon and small intestine of rats. Lab. Investig. (in press).

Ward, J. M., Yamamoto, R. S., and Brown, C. A.: Pathology of intestinal neoplasms and other lesions in rats exposed to azoxymethane. J. Nat. Cancer Inst. 51: 1029-1039, 1973.

Ward, J. M., Yamamoto, R. S., and Weisburger, J. H.: Cellulose dietary levels and azoxymethane-induced intestinal cancer. J. Nat. Cancer Inst. 51: 713-715, 1973.

Weisburger, J. H.: Chemical carcinogenesis in the gastrointestinal tract. In Seventh National Cancer Conference Proceedings, American Cancer Society, Inc., 1973, pp. 465-473.

Weisburger, J. H.: Chemical carcinogens and their mode of action in colonic neoplasia. Dis. Colon Rectum 16: 431-437, 1973.

Serial No. NCI-4619
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Metabolic Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mode of Action of Chemical Carcinogens -- Studies on the Metabolism of Chemical Carcinogens

Previous Serial Number: Same

Principal Investigator: Preston H. Grantham, M.S.

Other Investigators: Letitia C. Mohan, B.A., Thirayudh Glinsukon, Sc.D., Timothy Benjamin, B.S., Elizabeth K. Weisburger, Ph.D., Jane Idoine, B.S.

Cooperating Units: None

Man Years:

Total	3.7
Professional	1.9
Other	1.8

Project Description

Objectives:

The aim of this research is to gather data relevant to the etiology of neoplasia at the molecular level. To this end chemical carcinogens, especially those of the aromatic amine type, are being utilized. Their metabolism and interaction with host tissues and specific targets are studied. Simplified yet realistic model systems are devised in order to gain an understanding of the fundamental events operating in chemical carcinogenesis. Specific inhibitors and accelerators of the carcinogenic process are applied as tools to develop information on and permit discrimination between biochemical events directly involved in the neoplastic change and those representing other reactions.

Methods Employed:

Biochemical and pharmacological techniques are applied to determine the metabolism of N-hydroxy-N-2-fluorenylacetamide and related compounds in various animal species. This includes isolation and purification of enzyme systems concerned with certain metabolic steps.

Procedures are developed and applied for the separation, purification and analysis of macromolecular constituents such as DNA, RNA and proteins from tissues of animals treated with chemical carcinogens and from control animals.

The metabolism of chemical carcinogens and related compounds, and interaction with host targets are examined *in vivo* and *in vitro* in the presence of various chemicals or antibiotics which affect the carcinogenic process.

Major Findings:

1. Metabolism of *p*-hydroxyacetanilide under acute and chronic conditions. ^{3}H-*p*-Hydroxyacetanilide was prepared biosynthetically by injecting rats with ^{3}H-acetanilide. The urines were collected and the *p*-hydroxyacetanilide was isolated by DEAE-cellulose column chromatography. A metabolism study was done in controls fed an ordinary diet and animals which had been prefed unlabeled *p*-hydroxyacetanilide for 4 weeks. There was very little difference in the percent of labeled material excreted in the urines over a 24 hour period from control and prefed groups, respectively. However, there was an increase in the glucuronide fraction in the prefed group. Although *p*-hydroxyacetanilide is presumed to act as a sulfate trap, the amount of radioactivity excreted in the sulfate fraction was the same with each group. Likewise, water soluble metabolites were about the same. By means of paper and column chromatography these water soluble metabolites of *p*-hydroxyacetanilide were investigated further. One metabolite which was isolated and identified was found to be 3[5-acetamido-2-hydroxyphenyl)thio]-N-acetylalanine. By means of mass spectrometry and NMR data it was proved that this metabolite is the same as one of the water soluble metabolites of acetanilide which we reported on last year. The levels of certain important metabolic enzymes were determined in the livers of control rats and those prefed *p*-hydroxyacetanilide. Since the levels of glucuronides excreted were increased, it seemed reasonable that there should be an effect on glucuronyl transferase, the enzyme responsible for formation of these conjugates. These studies showed that prefeeding rats with *p*-hydroxyacetanilide for 4 weeks led to a three-fold increase of glucuronyl transferase activity in the liver microsomes over that in the levels of rats which had been fed a control diet. As a corollary, glucuronyl transferase activity was determined in the livers of rats which had been prefed FAA plus *p*-hydroxyacetanilide or N-OH-FAA plus *p*-hydroxyacetanilide or the carcinogens FAA or N-OH-FAA alone. It was found that in all cases prefeeding any one of the compounds or the mixtures led to an increase in glucuronyl transferase activity in the liver microsomes.

2. Metabolism of 6-aminochrysene as followed by 5,6-^{14}C-6-aminochrysene. Studies are continuing on the metabolism of 6-aminochrysene which has been suggested for treatment of breast cancer. As reported last year, a synthesis of ring-labeled 6-aminochrysene was devised in order to lend more certainty to our metabolism studies. Using the ring-labeled aminochrysene, it was found that only a small amount of compound was excreted into the urine after intraperitoneal injection. Excretion in the feces was somewhat higher indicating that the compound was absorbed very slowly from the peritoneal

cavity. Confirming the studies which had been done with the ^{3}H-labeled material, the glucuronide of 6-aminochrysene was the chief biliary metabolite. However, no N-glucuronide could be found in the feces indicating that transformation had occurred during passage through the intestinal tract. Studies on the metabolism of 6-aminochrysene excreted in the bile, urine and feces are continuing. 6-Acetylaminochrysene appears to be one of these metabolites. However, two hydroxylated compounds derived from 6-acetylaminochrysene, namely 12-hydroxy-6-acetylaminochrysene and N-hydroxy-6-acetylaminochrysene could not be identified as metabolites of 6-aminochrysene.

3. Attempts to synthesize the N-glucuronide of 6-aminochrysene. In view of the fact that the N-glucuronide was the chief biliary metabolite, several methods were employed in an attempt to synthesize this compound. Although the glucuronide of aniline was made very easily by reported methods, the N-glucuronide of 6-aminochrysene could not be obtained. A very small amount of the compound, however, was obtained by isolation from the bile and characterized by thin layer chromatography and color reactions with the naphthoresorcinol reagent. Further methods of synthesis are being explored.

4. *p*-Hydroxyacetanilide. Some other enzymes were also studied in the livers of rats which had been prefed with *p*-hydroxyacetanilide. In many cases aryl hydrocarbon hydroxylase is involved in detoxification of foreign compounds by formation of hydroxy derivatives which are then more readily conjugated and excreted. There are also reports that deacetylase is an important enzyme in the metabolism of some derivatives of aromatic amines. Sulfotransferase is the enzyme responsible for conjugation of aromatic compounds with sulfate to form sulfate esters which can be excreted. However, all the studies showed that neither of these enzymes was increased to any extent by prefeeding *p*-hydroxyacetanilide.

5. Metabolism of 6-acetylaminochrysene-$^{H}3$. 6-Acetylaminochrysene-$^{H}3$ was synthesized from 6-aminochrysene-$^{H}3$. A preliminary metabolism study of this compound showed that after a single i.p. injection approximately 4% of the dose was excreted in the urine in a 48-hour period. This was further subdivided as follows: free metabolites, 6%; glucuronides, 10%; sulfate conjugates, 50% of urinary radioactivity. At least 2 metabolites could be separated in the glucuronide conjugate fraction and 3 in the sulfate conjugate fraction after acid hydrolysis. The compound was absorbed very poorly from the intraperitoneal cavity.

6. Metabolism of FAA in mice. Dr. Anna Goldfeder of Columbia University has developed the X-GF strain of mouse which is extremely resistant to the carcinogenic effect of 2-fluorenylacetamide. These mice can be fed high doses for a lifetime and still not develop any of the usual tumors seen with this carcinogen. On the other hand, other strains of mice such as the NIH-Swiss developed various types of tumors, especially of the liver, after feeding FAA. Therefore, a metabolism study was done with X-GF mice provided by Dr. Goldfeder to determine whether there was any outstanding difference in metabolic pattern to account for the lack of carcinogenic effect. After a single dose of labeled FAA, X-GF mice excreted approximately the same

percentage into the urine and feces as did the NIH-Swiss mice. Fractionation of urinary metabolites showed that the only difference was that X-GF mice had twice the percent of water soluble metabolites as did the NIH-Swiss. In all other categories the metabolic pattern was quite similar. Within the glucuronide fraction which accounted for about 35% of a dose, there was no startling difference in percent of labeled metabolites excreted. Of the detoxification products the NIH-Swiss mice excreted about 20% of the dose as the glucuronide of 7-hydroxy-FAA while the X-GF mice excreted approximately 26% in this manner. The other detoxification products were very similar in percentage. The only remarkable difference was that the NIH-Swiss mice excreted twice as much N-OH-FAA as did the X-GF, namely 3.6 versus 1.5%. It has been assumed that the carcinogenic response of a species can be equated to some extent with the formation and excretion of the N-hydroxy derivative. This small difference is not enough to explain the very outstanding difference in long term response of the two mouse strains. There obviously are other factors which play a role and which should be investigated further. The X-GF mice excreted a hitherto unknown metabolite in the sulfate fraction, and attempts are being made to isolate and identify this material.

7. Study of water soluble metabolites of FAA. In view of the experience we have gained in identification of water soluble metabolites of acetanilide and *p*-hydroxyacetanilide, we have returned to the problem of the water soluble metabolites of FAA. XAD-2 Amberlite resin columns are being used to separate metabolites from naturally occurring salts and other natural constituents of urine. Previously these materials had complicated the use of thin layer chromatography and mass spectrometric analysis. This investigation will continue with the hopes of determining these water soluble metabolites which may be mercapturic acid conjugates.

8. *In vitro* study with liver cytosol from animals administered 2,4-toluenediamine. Many investigators have reported that liver cytosol is involved in the acetylation of amines, both foreign and endogenous compounds. In view of our proposed studies on the metabolism of 2,4-toluenediamine, an *in vitro* study with this compound is being undertaken. Liver cytosol with labeled acetyl co-enzyme A plus 2,4-toluenediamine as an acceptor will be studied to determine the effects of this cellular constituent on the process of acetylation. These studies have just begun and will continue.

Significance to Biomedical Research and the Program of the Institute:

By the utilization of certain chemical carcinogens, the molecular mechanism of steps leading to cancer is being explored. If the entire process is viewed as a series of steps from the primary interaction between an agent and a crucial cellular constituent or constituents, followed by multiplication of abnormal cells which in turn can undergo further transformations, there are a number of points where one could prevent or even reverse such interactions. Thus, the ultimate aim of these studies is to comprehend fully the sequence of complex reactions leading to cancer and eventually to be in a position to prevent them under realistic conditions in man.

Proposed Course of Project:

The program of elucidating the biochemistry of carcinogenesis, our eventual goal, is a continuing, long-term effort. Certain promising leads which have been discussed in this account will be followed up in further experiments.

Publications

Grantham, P. H., Mohan, L. C., Weisburger, E. K., Fales, H. M., Sokoloski, E. A., and Weisburger, J. H.: Identification of new water-soluble metabolites of acetanilide. Xenobiotica (in press).

Grantham, P. H., Weisburger, J. H., and Weisburger, E. K.: Effect of the antioxidant butylated hydroxytoluene (BHT) on the metabolism of the carcinogens N-2-fluorenylacetamide and N-hydroxy-N-2-fluorenylacetamide. Food Cosmet. Toxicol. 11: 209-217, 1973.

Mohan, L. C., Grantham, P. H., Weisburger, E. K., Weisburger, J. H., and Idoine, J. B.: Mechanisms of the inhibitory action of *p*-hydroxyacetanilide on carcinogenesis by N-2-fluorenylacetamide or N-hydroxy-N-2-fluorenylacetamide Cancer Res. (in press).

Poirier, L. A. and Weisburger, J. H.: N-Hydroxylation and carcinogenesis. Proc. 5th Int. Congr. Pharmacol. 2: 150-163, 1973.

Poirier, L. A. and Weisburger, J. H.: Enzymic reduction of carcinogenic aromatic nitro compounds by rat and mouse liver fractions. Biochem. Pharmacol (in press).

Weisburger, J. H. and Weisburger, E. K.: Biochemical formation and pharmacological, toxicological and pathological properties of hydroxylamines and hydroxamic acids. Pharmacol. Revs. 25: 1-66, 1973.

Serial No. NCI-4620
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Metabolic Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mode of Action of Chemical Carcinogens -- Chemical Investigations

Previous Serial Number: Same

Principal Investigator: Elizabeth K. Weisburger, Ph.D.

Other Investigators: Thirayudh Glinsukon, Sc.D. and Timothy Benjamin, B.S.

Cooperating Units: Analytical Chemistry Unit, CMT, NCI
Laboratory of Chemistry, NHLI
Viral Carcinogenesis Branch, NCI

Man Years:

Total	:	1.0
Professional	:	0.7
Other	:	0.3

Project Description

Objectives:

In order to gain an understanding of the etiology of cancer, chemical studies are performed on the properties of certain carcinogens. Model studies on the reactions of chemical carcinogens with potential targets are performed.

Methods Employed:

Properties of chemicals acquired or synthesized for the bioassay of chemical carcinogens are investigated to determine the purity of the materials. In model experiments the reaction of certain of these agents, particularly their primary and ultimate reactive forms with select potential targets are studied. The materials produced are made available for related studies on the biochemistry and biology of the carcinogenic process. Methods for purification and analysis of various chemicals are applied.

Major Findings:

During the past year this project continues to prepare reference materials for metabolism studies on carcinogens or co-carcinogenic analogs. Many possible metabolites of the industrial intermediate 2,4-toluenediamine have been synthesized for use in a metabolism study (Project 4619). Various carcinogens which are unavailable commercially are being synthesized also for the use of other members of the Branch (Project 4618).

Significance to Biomedical Research and the Program of the Institute:

The studies performed are a necessary adjunct to the broad programs on the mechanism of action of chemical carcinogens pursued in this Branch.

Proposed Course of Project:

This project will continue along the same lines as previously.

Honors and Awards

Thirayudh Glinsukon: Post-doctorate fellowship from the Fogarty International Center

Publications

Rhim, J. W., Weisburger, E. K., and Weisburger, J. H.: Evaluation of an *in vitro* assay system for carcinogens based on prior infection of rodent cells with non-transforming RNA tumor virus. J. Nat. Cancer Inst. (in press).

Weisburger, E. K.: Industrial cancer risks. In Sax, N. Irving (Ed.): Dangerous Properties of Industrial Materials. 4th Edition, New York, Reinhold (in press).

Serial No. NCI-4542
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Analytical Chemistry Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Chemistry of N-Nitroso Compounds

Previous Serial Number: Same

Principal Investigator: Larry K. Keefer, Ph.D.

Other Investigators: Peter P. Roller, Ph.D., Elizabeth K. Weisburger, Ph.D., Jerrold M. Ward, D.V.M., Ph.D., Jerry Rice, Ph.D., Sewa Rama Joshi, D.V.M., Ph.D., Walter Zielinski, Jr., Ph.D., Yuki Oshiro, Ph.D., and Gary M. Muschik, Ph.D. (Frederick Cancer Research Center); George Olah, Ph.D. and Daniel T. Donovan (Case Western Reserve University); William Lijinsky, Ph.D. (Biology Division, Oak Ridge National Laboratories); Kenneth Palmer, Ph.D. (USDA, Albany, California)

Cooperating Units: Metabolic Studies Section, CMT, NCI, Perinatal Carcinogenesis Section, EXP, NCI, Normal Volunteer Program.

Man Years:

Total	1.0
Professional	0.9
Other	0.1

Project Description

Objectives:

(1) To investigate mechanisms by which metal ions might promote the formation of N-nitroso compounds in the environment. (2) To study mechanisms of metabolism of and carcinogenesis by N-nitroso compounds using the deuterium isotope effect. (3) To study the reaction of N-nitroso compounds with protonating agents and other electrophilic species, making inferences where possible regarding the significance to carcinogenesis research of the reversible and irreversible chemical changes that ensue. (4) To synthesize possible metabolites of certain N-nitroso compounds for comparison of their physical, chemical, and biological properties with those of the parent procarcinogens.

Methods Employed:

In addition to the standard methods of synthetic and mechanistic chemistry, considerable use has been made of the deuterium isotope effect as a pharmacological probe. This method has not been widely exploited elsewhere, and it is possible that this project may in the long run demonstrate the technique's general utility in pharmacological investigations.

Major Findings:

(1) In collaboration with Dr. George Olah and Mr. Daniel Donovan of the Case Western Reserve University, three distinct degradation pathways which dialkylnitrosamines may undergo upon protonation in superacid medium have been characterized. Under a variety of conditions, the major pathway appears to be a disproportionation to HNO and iminium ions (protonated Schiff's bases of primary amines and carbonyl compounds). With nitrosamines bearing N-substituents corresponding to the more stable carbenium ions, the O-protonated compounds apparently suffer S_N1 ionization to diazotic acid and a cation capable of alkylating nucleophilic centers in the medium. The third pathway, simple denitrosation to the corresponding dialkylamine, is observed only under certain conditions. A case has been developed to suggest a) that these reactions are conceivable in vivo and b) that some of the known metabolic properties of dialkylnitrosamines can be explained much better on the basis of these reactions as metabolizing paths than of the generally accepted mechanism of dialkylnitrosamine metabolism. (2) In a parallel study, certain protonated nitrosamines presumed to be intermediates in the above rearrangements have been isolated as stable, crystalline salts. The base:acid ratios of these adducts may be either 1:1, as in the perchloric acid salt of dimethylnitrosamine, or 2:1, as with the hexafluorophosphoric acid-nitrosopyrrolidine compound. An X-ray crystallographic attempt is underway to determine the bond types involved in the 2:1 complexes, in collaboration with Dr. Kenneth Palmer of the USDA Laboratory in Albany, California. (3) Several derivatives of methyl-hydroxymethylnitrosamine have been synthesized, including the acetate ester and several alkyl ethers. Hydrolysis of these materials has been studied under a variety of conditions and found to yield the products expected of the enzymatic C-hydroxylation step presumed to be responsible for dimethylnitrosamine metabolism in vivo (nitrogen gas and a methylating species). Ultraviolet kinetic studies suggest that the parent compound's half-life is at most 10 minutes after generation by cleavage of the acetate ester using hydrolysis with hog liver esterase. The acetate's carcinogenic properties are being studied both classically and transplacentally by Drs. Rice and Joshi of the Perinatal Carcinogenesis Section, and in vitro transformation tests will be begun shortly by Drs. Weisburger and Ward in the Metabolic Studies Section. The hydroxymethyl compound's ability to alkylate nucleic acids will be determined at the Frederick Cancer Research Center. (4) It has been found that metal ions can promote the nitrosation of secondary amines. Several possible mechanisms for this promotion are being considered, each of which could be important determinants of the environmental distribution of N-nitroso compounds. (5) The relative abilities of dimethylnitrosamine (DMN) and its full

deuterium-labelled analog (DMN-d_6) to alkylate proteins and nucleic acids after administration to rats have been studied in detail in collaboration with Dr. Yuki Oshiro of the Frederick Cancer Research Center. The protium labelled material (DMN) was 2.7 times as effective a methylating agent for nucleic acids of liver (a target organ for this carcinogen), but only 1.4 times as effective in the lung (a non-target organ). It can be inferred that the methylation mechanisms in the two organs are not identical; in view of the wide array of significantly different isotope effects observed in the methylation of macromolecules in the organs investigated (ranging between the extremes of 1.0 and 2.7), it seems likely that at least two different methylation mechanisms are simultaneously operative and that their relative effectiveness changes markedly from one organelle type to another.

It has generally been assumed that both the metabolism and carcinogenicity of DMN are initiated by an enzymatic C-hydroxylation step, with a sequence of rearrangements then leading very rapidly to both methylation and formaldehyde production. If this is true, then both of these processes should proceed with isotope effects very similar to that of the enzymatic hydroxylation step, since the latter transformation, as well as being rate-determining, is the only step involving carbon-hydrogen bond cleavage. In contrast to the large isotope effect found on in vivo methylation of liver nucleic acids and proteins, however, DMN and DMN-d_6 were converted to formaldehyde at the same rate by liver microsomal preparations. Unless a fundamental change in the DMN metabolizing enzyme system occurs during microsome preparation, this result indicates that formaldehyde and the biological methylating agents are produced by quite different mechanisms.

An effort is underway to interpret these results in terms of the mechanism(s) of carcinogenesis by DMN. If enzymatic C-hydroxylation is the "rate-determining step" in the malignant transformation, the DMN might be predicted to act as a more effective carcinogen than DMN-d_6. That this is the case has recently been demonstrated, in accord with expectation based upon this mechanism of action.

Significance to Biomedical Research and the Program of the Institute:

With considerable public attention focused on the possibility that N-nitroso compounds might be responsible for some human cancer, collection of data on the chemical, physical, and biological properties of this hitherto poorly understood class of compounds is an urgent matter. Certain aspects of the studies reported here may aid in developing methods for predicting and analyzing the environmental distribution of these potential carcinogens, while other aspects are aimed at shedding light on the biochemical mechanisms by which these compounds exhibit their untoward effects.

Proposed Course of Project:

The studies described above will be completed and published, and the important hypotheses and implications they suggest will be investigated as

appropriate. In addition, new studies will be initiated. One program planned for the near future, for example, is the synthesis and characterization of spin-labelled N-nitroso compounds, for use as potential probes for the investigation of precovalent interactions these carcinogens might undergo with the biological matrix. Attention will also be given to the synthesis of some nitrosamino acids potentially useful for the isolation of nitrosamine-metabolizing enzymes.

Honors and Awards

Larry K. Keefer:

Member, Carcinogen Metabolism and Toxicology Segment, DCCP, NCI

Member, Information and Resources Segment, DCCP, NCI

Member, Chemical Selection Committee, DCCP, NCI

Member, Cancer Research Safety Committee, NCI

Invited Speaker, "Deuterium Isotope Effect on the Biological Properties of Dimethylnitrosamine", University of Minnesota, Department of Medicinal Chemistry, May, 1974.

Invited Speaker, Third IARC Meeting on the Analysis and Formation of N-Nitros Compounds, Lyon, France, October, 1973.

Publications

Keefer, L. K. and Roller, P. P.: N-Nitrosation by nitrite ion in neutral and basic medium. Science 181: 1245-1247, 1973.

Roller, P. P. and Keefer, L. K.: Catalysis of nitrosation reactions by electrophilic species. Proceedings of the Third Meeting on the Analysis and Formation of N-Nitroso Compounds, Lyon, France (in press).

Serial No. NCI-4547
1. Carcinogen Metabolism and Toxicology Branch, Carcinogenesis, DCCP
2. Bacterial Metabolism Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Laboratory Study of the Etiology of Colonic Cancer

Previous Serial Number: None

Principal Investigator: Sidney J. Silverman, Ph.D.

Other Investigators: Milton W. Slein, Ph.D., Mary J. Wilson, M.S., and A. W. Andrews (Litton Bionetics, Inc.)

Cooperating Units: Project 7, FCRC (Litton-Bionetics, Inc.)

Man Years:

Total	3.5
Professional	2.5
Other	1.0

Project Description

Objectives:

(1) Study the metabolism and physiology of intestinal bacteria in relation to the production, activation, or inactivation of carcinogens. (2) Develop *in vitro* assays for mutagenesis for use as screening tests for carcinogens.

Methods Employed:

The degradation of cholic acid by several species of anaerobic intestinal bacteria is studied. The products of bacteria metabolism are identified by chemical and chromatographic procedures and their mutagenic or co-mutagenic activity is determined by *in vitro* methods. Mixed cultures are also to be studied.

Major Findings:

Strains of *Bacteroides*, *Fusobacterium*, and *Peptostreptococcus* isolated from a patient with adenomatous intestinal polyps were selected for study because of their presence in large numbers in the feces of this patient. Growth in the presence of 0.25 mM of cholic acid resulted in the production of several products by *Bacteroides* and *Fusobacterium*. Among the identified metabolites,

deoxycholic acid and 7-keto-deoxycholic acid were most prominent. However, two strains of *Peptostreptococcus* showed no significant activity on cholic acid, deoxycholic acid, or 3α, 12α - dihydroxy - Δ - 8-14 - 5β cholenic acid. Attempts failed to induce enzyme activity by serial passage of the organisms in a medium containing bile acids. A semi-defined medium for use in metabolic studies was devised so that the effect of various additions on bacterial activity could be studied.

The host-mediated assay of Legator and Malling and the *in vitro* mutagenesis assay of Ames were investigated as assay methods for screening bacterial cultures for the presence of possible carcinogens. Although the response of individual mice was quite variable in the Legator host-mediated assay, with numbers of animals sufficient for statistical analysis the method is satisfactory. Thirty-six bile acids or derivatives were tested for mutagenesis or for ability to enhance mutagenesis of N-methyl-N'-nitro-N-nitrosoguanidine. No activity was observed.

Significance to Biomedical Research and the Program of the Institute:

The studies are an attempt to obtain laboratory support for the epidemiological evidence linking diet and intestinal microflora to the occurrence of large bowel cancer. Proof of this cause-effect relationship and the metabolic pathways that lead to the production of carcinogens, promoters, or co-carcinogens should aid in the control of this form of cancer - one of the most prevalent in the United States.

Proposed Course of Project:

Further studies will concentrate on the metabolism of bile acids by mixed bacterial suspensions in an attempt to more closely duplicate the intestinal environment. Methods will be improved for the more positive identification of metabolic products and determination of their mutagenic or co-mutagenic activity.

Honors and Awards

Sidney J. Silverman:

Acting Director, Colon Cancer Segment, Carcinogenesis, NCI

Observer-Member, Colon-Rectum Cancer Advisory Committee to National Large Bowel Cancer Project

Publications

Berg, J. W., Howell, M. A., and Silverman, S. J.: Dietary hypothesis and diet-related research in the etiology of colon cancer. Health Services Report 88: 915-924, 1973.

SUMMARY REPORT

CHEMISTRY BRANCH

July 1, 1973 through June 30, 1974

"Plans, develops and conducts a research program designed to (1) clarify the molecular biology of carcinogenesis; (2) elucidate the fundamental nature of the interactions of carcinogenic agents, especially chemical, with biological systems in the induction of cancer; (3) define those environmental and endogenous factors which relate to and modify the carcinogenic process; and (4) clarify the metabolic regulatory processes which are related to carcinogenesis."

The goal of the Chemistry Branch is to understand the molecular events of carcinogenesis with the view toward identifying susceptible populations and preventing human cancer. The research program is designed to understand the molecular basis by which carcinogenic agents cause malignant transformation and identify and characterize those exogenous and endogenous factors involved in carcinogenesis. The Branch seeks to clarify the metabolic interaction of exogenous agents with internal factors in the living organism at the molecular, cellular, and host levels and seeks to understand the consequences of these interactions in terms of cell regulation and carcinogenesis.

Cancer is a disease in which the expression of genetic information is altered to give the tumor phenotype. This altered phenotypic expression may be due to genetic or epigenetic events induced by either xenobiotic or endogenous factors which result in altered patterns of gene control. The central aim of the Branch is to understand how exogenous carcinogens and endogenous factors are processed by enzymatic mechanisms, how the carcinogen is converted to active forms and the nature of the interaction between the latter and the gene active system. Understanding is sought in how this initial interaction results in the modifications with characterize malignant transformation. The aim of these studies is modification, elimination, or prophylaxis of the carcinogenic response in the human population. Senior staff of this Branch is also responsible for the scientific direction of the Chemistry and Molecular Carcinogenesis Segment.

Molecular Carcinogenesis Section - *"Studies (1) the metabolic effects on tissues and cells resulting from their exposure to chemical, physical, and viral carcinogens and the relationship between these effects and malignant transformation; (2) the functional and metabolic consequence of carcinogen-macromolecule interaction."* This Section studies the molecular events of malignant transformation induced by chemical carcinogens, in particular those of the polycyclic hydrocarbon class. The primary focus of these studies is to understand the enzymatic conversion of carcinogens to either detoxification forms or to the active carcinogenic metabolite. In parallel studies the interaction of the active metabolite with the relevant cellular receptor has been investigated. During man's evolution he has been constantly exposed to foreign chemical compounds (including carcinogens) and has developed metabolic systems for their detoxification and elimination.

These systems primarily involve microsomal cytochrome P-450 mixed function oxygenases, but also include epoxide hydrases and conjugating enzymes. Thus the vast majority of foreign compounds are processed by these enzyme systems. The mixed function oxygenase is influenced by a variety of environmental factors such as exposure to drugs, pesticides or carcinogens, and is also influenced by the nutritional and hormonal state of the animal. The age, sex and genetic makeup are also determinants of enzyme activity. Work in the laboratories of this Branch provided the key studies which showed this enzyme system to be responsible for the activation of procarcinogens to their ultimate carcinogenic form. Thus it was shown that with some hydrocarbons mixed-function oxygenase was responsible for both the toxic and carcinogenic effects. This new and important understanding of polycyclic hydrocarbon action was derived in this laboratory from five lines of evidence: (1) the toxicity of benzopyrene (BP) parallels level of enzyme in the cell; (2) the enzyme system catalyzes the formation of BP-DNA complexes; (3) the discovery of an inhibitor of the enzyme system (7,8-benzoflavone) which prevents BP cytotoxicity; (4) the inhibitor of the enzyme prevents the binding of BP to DNA, RNA and protein; and (5) the inhibition of the enzyme system reduces dimethylbenzanthracene (DMBA) tumorigenicity by 90%.

A definition of the role of the enzyme system in human carcinogenesis by chemicals is a primary goal of our current research. Work in this Branch had previously demonstrated that the enzyme was present and measurable in human peripheral lymphocytes. More sensitive methods to detect the enzyme in easily obtainable human tissues such as blood are now being actively developed. The goal is to do the assay with a small enough volume of blood to permit the screening of human populations. In addition methods have been developed for the isolation and characterization of metabolites of polycyclic hydrocarbons (see below), which will be used for the analysis of metabolism of polycyclic hydrocarbon in human tissue.

One of the major difficulties in understanding polycyclic hydrocarbon carcinogenesis has been the analysis and quantitation of inactive and active metabolites formed from the polycyclic hydrocarbons. These methods in the past have been laborious and so inaccurate as to make quantitation impossible. In the last year a milestone advance in this methodology has been made in this laboratory which will enable all of the workers in the field of polycyclic hydrocarbon carcinogenesis to take steps forward towards understanding this process. This advance is based on the use of high pressure liquid chromatography which can accurately, clearly, and quantitatively separate ten metabolites of benzopyrene in a short period of time (40 minute This technique is now being applied to the study of tissue, species, and genetic differences in metabolism. With this technique tissue susceptibilit to carcinogens can be compared and correlated with unique metabolic profiles thereby permitting the identification of those metabolites which are detoxification producers and those metabolites which are the active forms of the carcinogens.

In another part of the program these laboratories have studied the metabolism of benzopyrene epoxide and are partially on the way towards developing an assay method for the determination for benzopyrene epoxide hydrase which

may be a key enzyme in converting the potent carcinogenic intermediate benzopyrene epoxide to the inactive dihydrodiol. The level of this enzyme may be uniquely important to carcinogenesis and an accurate assay for its activity would be most useful.

In another study, the anti-tumorigenic effects of a variety of benzoflavone analogues have been determined, examining both their activity as stimulators or inhibitors of aryl hydrocarbon hydroxylase and of carcinogenesis. A large number of compounds which can inhibit or stimulate the mixed function oxygenases have been found. Plans to study their ability to change the metabolic profile of metabolites are underway. A class of anti-radiation compounds is studied in collaboration with Dr. Daniel Klayman at Walter Reed Hospital. It was found that some of these compounds have unique anti-tumorigenic effects, and their mechanism is under investigation.

As part of the studies on the regulation of mixed-function oxygenases, a liver cell culture system was developed for the study of aryl hydrocarbon hydroxylase. It was found that in a cloned cell system substantial variation in both basal and induced hydroxylase activity developed during growth in cell culture, indicating a heterogeneity among different cells derived from the same parent. This heterogeneity may be very important in defining optimal conditions for the in vitro transformation of cells by polycyclic hydrocarbons.

Extraordinarily sensitive in vitro cell culture systems for the detection of minute amounts of enzyme inducers were developed in this laboratory. Thus the cell culture system can detect levels of inducers as low as 10^{-9} moles of compounds. This is a level of polycyclic hydrocarbon which can be found in some polluted water or in ordinary foods. The exquisite sensitivity of this method would permit the easy screening of materials in atmospheric pollutants for the inducers of microsomal mixed-function oxygenase systems. Mechanism studies with this enzyme system have shown that it can be induced by a block in protein systhesis followed by removal of the block. This suggests that the enzyme is regulated by a repressor mechanism with which the inducer interacts. These studies suggest that the regulation of this enzyme is controlled by RNA transcription from the gene and is regulated by both the presence of an endogenous repressor and the exogenous added inducer. In another study the regulation of aryl hydrocarbon hydroxylase and tyrosine aminotransferase in Reuber hepatoma cells in culture have been compared. These studies indicate similar mechanisms for the induction by steroids of the tranaminase and the hydroxylation of the hydroxylase. One of the key findings in this study was that of an interaction between these two induction mechanisms. Those cells exposed to the steroid inducers are much more inducible for the hydroxylase by the hydrocarbon. This shows a possible basis for the modulating effect of steroids on polycyclic hydrocarbon tumorigenesis and may relate to the well-known hormonal modulation of carcinogenesis.

In another study it was found that the preservatives butylated hydroxyanisole and butylated hydroxytoluene have effects on the hydroxylase enzyme system. Attempts are underway to correlate these effects in respect to the modulation of carcinogenesis by these agents.

Cell Growth Regulation Section - *"Studies (1) the biochemical factors controlling cell growth and differentiation and the nature of the mechanisms governing the phenotypic expression of genetic information; (2) oncogenic and non-oncogenic viruses and other environmental agents capable of inducing alterations in growth behavior and differentiation."* The primary goal of this Section is to define biochemically the tumor cell and in particular the nature of the molecules responsible for converting a normal cell to its malignant form. One approach is to examine certain classes of molecules known to be involved in protein synthesis. Transfer-RNA (tRNA) is known to function in the assembly of the proper amino acids into the growing protein chain. Another role which has been indicated for tRNA is that of the regulation of protein synthesis in terms of the initiation, elongation and termination of the growing protein molecule. The majority of these studies in the past have been with biochemical systems derived from microorganisms. Analysis of mammalian tissues has revealed the regular occurrence of amino acyl-tRNAs which recognize termination (nonsense) codons. If these amino acyl-tRNAs can suppress nonsense mutations, they may serve as a protective mechanism against mutagens which induce such nonsense mutations. These mutagenic agents may be also carcinogenic. When amino acyl-tRNAs from different organs were compared, variations in virtually all 20 amino acyl-tRNAs were found. The role of tRNAs in different tissues is being explored further through the manipulation of reticulocytes. These studies may provide a new understanding of the regulatory processes involved in carcinogenesis.

Viruses which transform cells into malignant forms have been used effectively to examine physiological processes involved in development of the malignant state. Cellular division, although necessary to the growth of a tumor, was shown to be unnecessary for the induction of metabolic changes accompanying malignancy, i.e., a cell can become potentially malignant without dividing.

Mutants of tumor viruses have been isolated, and cells infected with a mutant of Rous sarcoma virus act as tumor cells at one temperature and as normal cells at a higher temperature. The morphological change characteristic of malignant transformation can be separated from several biochemical changes which occur as a result of transformation, and the primary physiological change can be studied in the absence of complicating secondary changes. Cells in the process of transformation increase in size, and their density, as measured in dextran density gradients, decreases. These changes can be observed in the absence of DNA, RNA, or protein synthesis, and can be explained by an observed increase in intracellular water. An increase in intracellular water undoubtedly affects the activity of monovalent cations, which could in turn affect the regulation of cellular metabolis In fact, manipulation of the ionic environment of nontransformed and transformed cells demonstrated that glucose metabolism critically depended upon the concentration of monovalent cations. This transformation system offers the hope of a definition of the molecule responsible for the physiological manifestation of malignancy, and for the study of the sequence of metabolic events culminating in malignancy.

Nucleic Acids Section - *"Studies (1) interaction of chemical carcinogens and their analogs with nucleic acids and with enzymes regulating nucleic*

acid metabolism; (2) the effect of these agents on the subcellular organization of nucleic acid macromolecules." This Section is concerned with understanding the regulation and metabolism of nucleic acids with a view towards applying this information to our understanding of carcinogenesis. Carcinogens interact with RNA and with DNA. Major theories of chemical carcinogenesis suggest that the interaction with DNA is a key event and that the binding of chemical carcinogens with DNA is repairable by a group of DNA repair enzymes. Thus intimate knowledge of mechanisms of DNA replication and a cell's ability to repair damaged DNA is vital to understanding carcinogenesis.

In one area of investigation, work has continued on the study of the mechanism of DNA replication. Current results with bacterial systems confirm the "Okazaki" model of DNA synthesis, in which small fragments of DNA are individually synthesized prior to assembly. Furthermore, the number of independent polymerizing regions per replication fork varies in response to the temperature of incubation and the availability of thymine (in thymine requiring mutants). Under ideal growth conditions this number is two, that is, one per daughter strand per fork.

Secondly, the very sensitive assay for a cell's ability to repair damaged DNA (using Adenovirus-2 as the indicator genome in a host-cell reactivation system) that was developed in this laboratory has been used to test the repair capability of a variety of human cell lines. To date the following human cell lines have been assayed for ability to repair UV-damaged DNA: 10 normal strains, 13 Xeroderma pigmentosum strains from 4 different complementation groups showing varying degrees of impairment of their ability to repair UV-damaged DNA, 3 Fanconi's anemia strains, and 1 strain from a patient with Bloom's syndrome. The latter 3 strains showed normal repair in this assay. The same assay is also being used to measure and compare the ability of various human cell lines to repair damage to DNA caused by nitrous acid, psoralen plus light (365nm), 3-hydroxybenz(a)pyrene 4,5 epoxy benzpyrene (in the presence of Cu^{++} ion) and ^{60}C gamma rays.

A new research project is devoted to attempts to transform human cells, both normal and defective in repair mechanism.

Other studies in this Section continue to investigate the subcellular and molecular events that follow the infection of susceptible cells, cultured in vitro, with RNA tumor viruses. Work indicating that the plasma membrane of these cells is the major site to which the infecting viral RNA genome goes and in which this genome is transcribed to DNA will be published shortly. Further investigations of this process using enucleated cells are currently underway.

The Protein Section - *"Studies (1) the relationship of proteins to the carcinogenic process; (2) interactions between carcinogenic agents and proteins; (3) changes in proteins occurring as a result of or concomitant with the administration of carcinogenic agents; relates the genetic control of such proteins to inheritance in laboratory animals and man."* The Protein Section, as in the past, has been concerned with the development of better methods for the isolation and characterization of ribonucleic acids and

proteins. In order to provide a sound understanding of these substances, which regulate growth and function in normal and malignant cells, they must be isolated, separated and chemically defined. The goal of this Secti is to establish the means by which this can be accomplished, and to use this firm scientific background for studies in the nature of carcinogenesis.

Studies initiated during the previous year on methods for isolation of RNA have demonstrated that high concentrations of salt in the extraction fluids promote the association of heterogeneous nuclear RNA and proteins including nucleases. Such complexes behave in many respects like very high molecular weight RNA. Although these complexes can be dissociated in denaturing solutions, they may be minimized by the use of very low salt concentrations in the extraction fluids.

Recent studies from other laboratories have indicated that the protein composition of ribosomal subunits must be altered to permit attachment to the polysome. It has been known for many years that the 28S and 18S RNAs in ribosomes consist of two or more electrophoretically separable members. Studies have been started to determine whether conformational changes in the ribosomal RNAs result from such changes in the protein composition of the subunit.

Acrylamide monomers containing positively charged nitrogen (as choline) have recently become available. They may be incorporated into polyacrylamic gels producing a conventional gel which has the additional property of an ion exchanger. By various extensions of conventional electrophoresis and chromatography it has been possible to make crude fractionations of transfe RNAs. Although separations have been achieved superior to those obtainable by electrophoresis alone, it is premature to evaluate the utility of this technique. It has promise, however, as an easily applicable process for the total enumeration of transfer RNA species present in the tissues. Because transfer RNAs are thought to play important roles in control of translation, these analytical methods might be very important in the study of malignancy.

The studies started last year on the usefulness of the carbocyanine dye "Stains-all" as a phosphoprotein stain have been much extended. Conditions have been found for very sensitive demonstration of proteins conjugated with a variety of acidic groups including acid glycoproteins, sialic acid containing proteins, mucopolysaccharides, etc. In addition to being more sensitive than earlier techniques, the variety of colors produced helps to establish the identity of material under study. These findings have direct application to the current studies on the role of glycoproteins in surface membranes and should thus contribute directly to an improved understanding of the role of the surface membrane in carcinogenesis.

This laboratory has previously reported that denatured mouse satellite DNA could be resolved into two components by gel electrophoresis. During the past year it was confirmed that these two DNA fractions are the left and right strands of the mouse satellite DNA. Unlike other DNA specimens which were examined, the strand richer in G-C migrates more slowly than the A-T rich strand.

Serial No. NCI-4520
1. Chemistry Branch, Carcinogenesis, DCCP
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Biochemistry of Transformation of Cells by Avian Sarcoma Viruses

Previous Serial Number: Same

Principal Investigators: John P. Bader, Ph.D.

Other Investigators: Artrice V. Bader, Ph.D.

Cooperating Units: Virus Studies Section, Viral Oncology

Man Years:

Total	2.8
Professional	1.2
Other	1.6

Project Description

Objectives:

To delineate the basic biochemical defect responsible for the change to malignancy in Rous sarcoma virus-infected cells, and to determine the subsequent sequence of metabolic events resulting in the malignant state.

Methods Employed:

Cell culture, microscopic identification of cellular transformation, radioactive tracer techniques, isolation and purification of hyaluronic acid, polyacrylamide and agarose gel electrophoresis, electron microscopy, cell volume determinations, dextran density gradients.

Major Findings:

Several biophysical changes, in additions to morphological changes, occur in chick embryo cells after transformation by the Bryan "high titer" strain of Rous sarcoma virus (RSV-BH). Certain morphological changes observed in cells growing in monolayer are characteristic of transformation by RSV-BH, including intense vacuolization, rounding, and apparent enlargement. When transformed cells are suspended and measured, the average diameter and volume are larger

than nontransformed cells, and the density of RSV-BH transformed cells, as measured in density gradients of Dextran T 110, is lower than the density of nontransformed cells. These changes in biophysical parameters can be explained by the observed increase in intracellular water in RSV-BH transformed cells. Similar changes did not occur in cells transformed by another strain (Schmidt-Ruppin) of avian sarcoma virus.

Mutants of RSV-BH have been isolated which transform cells at 37° but not at 41°, although full cycles of virus reproduction proceed at both temperatures. The molecule directly responsible for transformation, therefore, is neither a structural component of the virion nor a substance involved in the synthesis of virion components.

The temperature-dependent morphological transformation induced by one of these mutants occurs without a requirement for simultaneous DNA, RNA, or protein synthesis, and the cellular changes in diameter, volume, and density, noted above, likewise occur in infected cells in the absence of DNA, RNA, or protein synthesis. These observations suggest that the transformation-molecule is in some way related to water and ion exchange, and studies are in progress to better define the factors involved.

Hyaluronic acid synthesis and the capacity for hexose (glucose, deoxyglucose, or glucosamine) uptake are higher in cells transformed by RSV-BH than in nontransformed cells. Cells infected with the mutant virus, and conditionally dependent for transformation, synthesize more hyaluronic acid and increase the capacity to take up hexose within a few hours after being shifted from 41° to 37°. These increases are inhibited by Actinomycin D or cycloheximide, demonstrating that new RNA and protein synthesis are required. Induction of increased hexose uptake and hyaluronate synthesis are markedly dependent upon the pH of the culture medium within the range 6.6 to 7.8. In contrast, the uptake of other nutrients including galactose, thymidine, uridine, and leucine, are essentially unaffected by differences in pH between 7.0 and 7.8, although uptake of all these nutrients increases during transformation. Induction of increased hexose uptake can occur in a simple salt solution containing glucose, and deletion of selected cations during incubation can prevent induction. Thus, a system for the study of the regulation of transcription in vivo has been developed, and factors inducing metabolic changes characteristic of the malignant state can be examined.

These observations suggest that cells in the process of transformation by RSV-BH take up water, probably accompanied by Na+. Increased H_2O-Na+ levels could then induce a sequence of changes (intracellular H_2O=Na+ → intracellular K+ → transcription → translation → ?) transforming the cell to the malignant form. Studies to examine specific possible changes within this sequence are in progress.

Significance to Biomedical Research and the Program of the Institute:

These findings encourage us to think that the delineation of the specific molecule responsible for malignancy induced by Rous sarcoma virus is within

sight. Such a determination would be a major step in defining the nature of cancer, and may be useful in devising programs for the control of cancer in man.

Proposed Course of Project:

To characterize more fully the morphological change in transformed cells; to search for the temperature sensitive molecule initially responsible for transformation in an attempt to define the specific biochemical defect responsible for malignancy in this system, and to delineate the sequence of metabolic events leading to the malignant state.

Serial No. NCI-4748
1. Chemistry Branch, Carcinogenesis, DCCP
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Synthesis and Characterization of the RNA of RNA-containing Tumor Viruses

Previous Serial Number: Same

Principal Investigators: John P. Bader, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total	:	0.6
Professional	:	0.2
Other	:	0.4

Project Description

Objectives:

To determine the molecular structure of the RNA of RNA-containing tumor viruses and to elucidate the intracellular site and mechanism of synthesis of viral RNA.

Methods Employed:

Cell culture, radioactive tracer techniques, density-equilibrium and sedimentation rate centrifugations, isolation and purification of nucleic acids, polyacrylamide gel electrophoresis.

Major Findings:

Our continued experimentations on the resolution of viral RNA by electrophoresis in polyacrylamide-agarose gels have demonstrated that aside from a single slowly migrating species (approx. 6-8 x 10^6 m.w.) which can be denatured to a more rapidly migrating form (approx. 3-4 x 10^6), all other observed RNA's can be explained by inadvertant enzymatic degradation of the larger forms.

Virions of avian leuko-sarcoma virus contain the denatured form of RNA, if the virions are harvested within minutes after completion. Incubation of these same virions at 37° to 40° for 1 hr results in viral RNA of the usual native form. Addition of various enzymes or enzyme inhibitors had little effect on the form of viral RNA observed in fresh harvests or on the transition after incubation. These changes were not found in virions of murine leukemia virus; only the larger native RNA was detected, even in virions harvested within three minutes after production. Kinetic experiments likewise failed to indicate a virion precursor to the native viral RNA. These differences between murine and avian viruses may help to elucidate the structural nature of the intravirion RNA of RNA tumor viruses.

Significance to Biomedical Research and the Program of the Institute:

The synthesis of viral RNA, its interaction with cellular organelles, its translation into viral structural and nonstructural proteins, and its packaging into complete virions, are all features of the viral reproductive process which are relevant to malignancy, but about which little is known. Analysis of these processes will help to give us a clearer understanding of the nature of carcinogenesis.

Proposed Course of Project:

To define the structure of viral RNA, to locate the site of viral RNA synthesis, to analyze the interaction of viral RNA with cellular organelles, and ultimately to describe the products of the viral genes responsible for tumorigenesis.

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Nonsense Codon Recognition in Mammalian Tissues

Previous Serial Number: Same

Principal Investigators: Dolph Hatfield, Ph.D., and M. Caicuts

Other Investigators: None

Cooperating Units: None

Man Years:

Total	2.0
Professional	1.0
Other	1.0

Project Description

Objectives:

To determine if purified mammalian AA-tRNAs which recognize nonsense codons can suppress nonsense mutations in a procaryotic and/or eucaryotic cell-free protein synthesis system using bacteriophage RNA as a source of messenger RNA.

Methods Employed:

AA-tRNAs which recognize nonsense codons have been purified from bovine liver by chromatography on BD-cellulose and reverse phase chromatographic columns I (Kelmers et al, J. Biol. Chem. 240: 3979, 1965) and V (Kelmers and Heatherly, Anal. Biochem. 44: 486, 1971). Bacteriophage RNA was extracted from R17 wild type, R17 amber and Qβ. Cell-free protein synthesis systems have been prepared from E. coli MRE 600 and from wheat germ (Roberts and Patterson, PNAS 70: 2330, 1973). Presently, a stimulation in the incorporation of labeled amino acids into protein in response to specific mRNAs is determined.

Major Findings:

An analysis of the AA-tRNAs in mammalian cells for recognition of nonsense codons has demonstrated the occurrence of several AA-tRNAs which recognize UAA, UAG and/or UGA (Hatfield, PNAS 69: 3014, 1972: Hatfield, Ninth Internatl. Congr Biochem., p201, 1973 [Abstract]). Each of these AA-tRNAs has been purified

extensively in order to add to a cell-free protein synthesis system containing a mRNA with an amber (UAG) or opal (UGA) termination signal.

Significance to Biomedical Research and the Program of the Institute:

A major unresolved question in biology is whether tRNA may play a role in cellular regulation and carcinogenesis. One approach to elucidating these problems is a study of the occurrence of suppressor tRNAs.

Proposed Course of Project:

Purified mammalian AA-tRNAs which recognize nonsense codons will be added to the cell-free protein synthesis systems (see above). If additional product is synthesized in response to an added AA-tRNA, the product will be characterized.

Serial No. NCI-4557
1. Chemistry Branch, Carcinogenesis, DCCP
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Chromatographic and Codon Response Differences in the Amino Acyl-tRNAs of Mammalian Tissues

Previous Serial Number: None

Principal Investigators: Dolph Hatfield, Ph.D., M. Caicuts and C. Robert Matthews*

Other Investigators: None

Cooperating Units: None

Man Years:

Total	:	2.2
Professional	:	1.2
Other	:	1.0

Project Description

Objectives:

To detect chromatographic differences in the AA-tRNAs of different mammalian tissues and to determine codon responses of isoaccepting species of AA-tRNAs from different mammalian tissues.

Methods Employed:

New Zealand white rabbits are treated with phenylhydrazine to induce reticulocytosis. Transfer RNA and AA-tRNA synthetases are subsequently isolated from rabbit liver and from reticulocyte rich blood. Chromatographic differences are detected by comparing labeled AA-tRNAs from liver (aminoacylated with [^{14}C] amino acid in the presence of rabbit liver AA-tRNA synthetases) to labeled AA-tRNAs from reticulocytes (aminoacylated with [^{3}H] amino acid in presence of rabbit reticulocyte AA-tRNA synthetases) on a reverse phase chromatographic column (RPC V; Kelmers and Heatherley, Anal. Biochem. 44: 486, 1971). Large scale preparation of labeled AA-tRNAs from liver and from reticulocytes are then resolved over a RPC V column and individual isoaccepting species of AA-tRNA

* A portion of this work was carried out when C. Robert Matthews (California Institute of Technology) was visiting the National Cancer Institute.

are pooled and assayed for codon recognition by the binding assay of Nirenberg and Leder (Science 145: 1399, 1964).

A similar study is being pursued with the chromatographic differences in bovine brain and liver which were previously observed in this laboratory (Hatfield et al., Cancer Res. 31: 697, 1971).

Major Findings:

Chromatographic differences were observed in the elution profiles of virtually all 20 AA-tRNAs of rabbit liver and rabbit reticulocytes (see also Smith and McNamara, Biochem. Biophys. Acta, in press). Codon responses of isoaccepting species of 18 AA-tRNAs from rabbit liver and reticulocytes have been determined. These studies have demonstrated that the levels of some isoaccepting species of AA-tRNA which recognize a specific codon are present in greater quantities in some tissues than in others (e.g., the seryl-tRNA which recognizes the codon UCG is more pronounced in liver than in reticulocytes).

Significance to Biomedical Research and the Program of the Institute:

A major unresolved question in biology is whether tRNA may play a role in cellular regulation and carcinogenesis. One approach to elucidating these problems is a study of the tissue specificity of AA-tRNAs.

Proposed Course of Project:

To determine if the levels of isoaccepting species of AA-tRNAs in a given tissue is related to the frequency of its usage in protein synthesis.

Serial No. NCI-4559
1. Chemistry Branch
Carcinogenesis, DCCP
2. Molecular Carcinogenesis
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Benzo[a]pyrene-4,5-Epoxide Hydrase

Previous Serial Number: None

Principal Investigators: Janet C. Leutz, Harry V. Gelboin, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.6
Professional:	0.3
Other:	0.3

Project Description

Objectives:

Development of an assay for benzo[a]pyrene-4,5-epoxide hydrase and determination of metabolic products formed from benzo[a]pyrene-4,5-epoxide (BPO).

Methods Employed:

High pressure liquid chromatography, column chromatography, spectrofluorometry U.V. spectral analysis, mass spectral analysis.

Major Findings:

This work was begun in November, 1973. The recently available metabolites of benzo[a]pyrene (BP), the K-region epoxide, BPO, and the K-region diol, trans-4,5-di-hydroxy-4,5-dihydro-benzo[a]pyrene have been characterized by fluoresence and U.V. spectroscopy respectively. The metabolism of BPO by liver microsomes from 3-methylcholanthrene-treated rats in the presence and absence of NADPH has been analysed by high pressure liquid chromatography.

Significance to Biomedical Research and the Program of the Institute:

Following metabolic activation, BP binds to DNA, RNA, and protein. It is currently thought that epoxide intermediates may be the activated form of BP which binds to macromolecules. The epoxide hydrase reaction is one of the major mechanisms for metabolism of BPO, hence it is important for removal of BPO from an animal system.

Proposed Course of Project:

Once the BP-epoxide hydrase assay has been developed, it will be possible to determine and compare epoxide hydrase activity in tissues including skin of strains of mice that are refracory and non-refractory to BP.

Serial No. NCI-4532
1. Chemistry Branch, Carcinogenesis, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Role of Microsomal Metabolism in the Malignant Transformation of Hamster Embryo Cells in Tissue Culture

Previous Serial Number: Same

Principal Investigator: James K. Selkirk, Ph.D.

Other Investigator: Harry V. Gelboin, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.6
Professional:	0.1
Other:	0.5

Project Description

Objectives:

Establishment of a system for the malignant transformation of clones of cells to study their metabolism of polycyclic hydrocarbons.

Methods Employed:

Cell culture; enzymology.

Major Findings:

We have found a different phenol in normal hamster cells that is not present in hamster liver and indicates differences in metabolic pathways.

Significance to Biomedical Research and the Program of the Institute:

Working with a homogeneous population as obtained in cell culture should magnify any differences one can find in a heterogeneous population as in skin or liver. This is with respect to relative amounts and molecular types of polycyclic hydrocarbon derivatives.

Proposed Course of Project:

Increase yield of metabolites to obtain sufficient material for characterization and determine if transformed hamster cells show the same new phenol.

Serial No. NCI-4556
1. Chemistry Branch, Carcinogenesis, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Role of Sulfhydryl Groups in Polycyclic Hydrocarbon Metabolism and Carcinogenesis

Previous Serial Number: Same

Principal Investigator: James K. Selkirk, Ph.D.

Other Investigators: Harry V. Gelboin, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.4
Other:	0.4

Project Description

Objectives:

To determine the relationship of sulfhydryl binding of carcinogens to the metabolism and carcinogenesis of polycyclic hydrocarbons. The binding of sulfhydryl compounds such as glutathione renders the conjugate water soluble. We plan to investigate the kinetics of binding of polycyclic hydrocarbons to glutathione in mouse skin.

Methods Employed:

Enzymology, spectrofluorometry, mouse skin tumorigenesis, and clorimetric analysis.

Major Findings:

A series of thiophosphoric esters and disulfides used as radiation agents reduce skin tumorigenesis in mice.

Significance to Biomedical Research and the Program of the Institute:

Conjugation of toxic substances to metabolites such as glutathione or glucuronic acid are common pathways for drugs and other foreign organic

chemicals taken into the living organism. If the lifetime of the active form of the carcinogen is determined by the rate of its conjugation to a nontoxic product, then acceleration of the reaction should reduce the half-life of the proximate carcinogen and consequently the formation of tumors.

Proposed Course of Project:

To determine the effect of these reagents upon the drug metabolizing enzymes and the metabolite pattern of Benzo[a]pyrene.

Serial No. NCI-4533
1. Chemistry Branch, Carcinogenesis, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Kinetics of Product Formation During Polycyclic Hydrocarbon Metabolism

Previous Serial Number: Same

Principal Investigator: James K. Selkirk, Ph.D.

Other Investigators: Harry V. Gelboin, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.2
Other:	0.3

Project Description

Objectives:

Determination of rate of formation of products. Determination of precursor-product relationship as well as the relationship between phenol and dihyrdodi i.e. hydration vs. dehydration of the various metabolites.

Methods Employed:

High-pressure liquid chromatography; fluorescence assay.

Major Findings:

MCA-induced liver microsomes metabolize BP to 3-hydroxy-BP at zero order kinetics for about 10 minutes as measured by fluorescence assay. At this time the metabolite profile is constant up to 30 minutes.

Significance to Biomedical Research and the Program of the Institute:

There are a number of similar products formed during metabolism of benzo[a]-pyrene. It is not yet known which of these derive from the activated carcinogenic state and which ones are from other intermediates. This new technique should allow rapid analysis during metabolism so molecular

activation can be observed. These separations are necessary in order to determine the relationship between metabolism and carcinogenicity.

Proposed Course of Project:

Incubations at shorter times will indicate the chronology of metabolite appearance and the preferential metabolic pathway will be determined.

Serial No. NCI-4534
1. Chemistry Branch, Carcinogenesis, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Separation of Polycyclic Hydrocarbon Metabolites by High-Pressure Liquid Chromatography

Previous Serial Number: Same

Principal Investigator: James K. Selkirk, Ph.D.

Other Investigators: Harry V. Gelboin, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.8
Professional:	0.4
Other:	0.4

Project Description

Objectives:

The rapid and quantitative separation of the known derivatives of carcinogenic polycyclic hydrocarbons. We are attempting to maximize the efficiency of separation by variance of column packing, solvent and temperature while maintaining molecular structure.

Methods Employed:

Dupont 830. High-Pressure Liquid Chromatograph.

Major Findings:

All labile functional groups (e.g. epoxy, hydroxyl) must be protected and must be able to be volatilized in order to utilize gas chromatography. In the case of polycyclic hydrocarbons, volatilizing temperatures are usually too close to the decomposition point to adequately separate polycyclic hydrocarbon derivatives. These compounds when injected can pass through this liquid chromatography system without decomposition. Eight metabolites of BP have been isolated and characterized by this system (Science in press).

Significance to Biomedical Research and the Program of the Institute:

The product profile of polycyclic hydrocarbon metabolism indicates some relationship between the various products formed and activity as a carcinogen. A rapid analytical technique will allow us to determine which metabolic route is the most related to carcinogenicity and which to detoxification. Rapidity of analysis will lessen the chance of oxidation or other non-metabolic breakdown product formation and give a more accurate picture of the system in its *in vivo* state.

Proposed Course of Project:

We propose to utilize this new technique to study comparative metabolite patterns in various animal and human tissues. Also we will continue to develop further solvent procedures to permit analysis of short lived intermediates, and reaction kinetics.

Publications

Selkirk, J.K., Croy, R.G. and Gelboin, H.V.: Benzo[a]pyrene Metabolites: Efficient and Rapid Separation by High-Pressure Liquid Chromatography. Science, 1974 (in press).

Serial No. NCI-4565
1. Chemistry Branch, Carcinogenesis, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Comparative Studies of Rodent Liver Metabolism of Benzo[a]-pyrene

Previous Serial Number: None

Principal Investigator: James K. Selkirk, Ph.D.

Other Investigators: Harry V. Gelboin, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.5
Professional:	0.2
Other:	0.3

Project Description

Objectives:

To measure the metabolite profile of enzo[a]pyrene in mouse, rat and hamster liver. Also, to compare control liver to Methycholanthrene-treated liver.

Methods Employed:

High-pressure liquid chromatography.

Major Findings:

While rat and mouse show relatively the same pattern, hamster liver shows more selective dihydrodihydroxy production. Induced liver shows marked increase in dihydrodihydroxy production.

Significance to Biomedical Research and the Program of the Institute:

Since there is a species susceptability to tumor formation by polycyclic hydrocarbons, it is important to determine which are the preferential metabolic pathways in susceptible and refractory species.

Proposed Course of Project:

We intend to determine if there are preferential metabolic pathway differences between rodent species.

Serial No. NCI-4561
1. Chemistry Branch,
Carcinogenesis, DCCP
2. Molecular Carcinogenesis
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Aryl Hydrocarbon Hydroxylase Induction and Tyrosine Aminotransferase Induction in Hepatoma Cells in Culture

Previous Serial Number: None

Principal Investigator: James P. Whitlock, Jr., M.D.

Other Investigators: Harry V. Gelboin, Ph.D.

Cooperating Units: None

Man Years:

Total:	0.6
Professional:	0.3
Other:	0.3

Project Description

Objectives:

Comparison of the mechanism of hydroxylase induction and transaminase induction in the Reuber (H35) cell strain.

Methods Employed:

Cell culture; enzymology.

Major Findings:

Microsomal aryl hydrocarbon hydroxylase is induced 25-fold by the polycyclic hydrocarbon benz[a]anthracene but is not induced by the steroid hormone dexamethasone. Soluble tyrosine aminotransferase is induced 12-fold by dexamethasone and 4-fold by benz[a]anthrene. Each enzyme requires similar inducer concentrations for induction, and their induction kinetics are similar. The induction of each enzyme requires RNA and protein synthesis; in each case the transcriptional and translational steps can occur independently. The two induction mechanisms are differentially sensitive to inhibitors of macromolecular synthesis. Simultaneous exposure to both inducers produces increases in both enzyme activities that are greater than those produced by either inducer alone. Each inducer acts at the transcriptional level to produce this synergistic effect.

Significance to Biomedical Research and the Program of the Institute:

An understanding of the regulation of hydroxylase activity is relevant to chemical carcinogenesis, drug metabolism, toxicology, and ecology.

Proposed Course of Project:

Further examination of the regulation of AHH in this hormone-responsive cell strain.

Serial No. NCI-4531
1. Chemistry Branch, Carcinogenesis, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Characterization of a Liver Cell Culture System for the Study of Aryl Hydrocarbon Hydroxylase (AHH)

Previous Serial Number: Same

Principal Investigator: James P. Whitlock, Jr., M.D.

Other Investigators: Harry V. Gelboin, Ph.D., Hayden G. Coon, Ph.D.

Cooperating Units: Laboratory of Cell Biology, NCI

Man Years:

Total:	0.4
Professional:	0.2
Other:	0.2

Project Description

Objectives:

Determination of the variability of AHH activity within a rat liver cell strain.

Methods Employed:

Cell culture, enzymology.

Major Findings:

There is substantial variation in basal and induced hydroxylase activity in clones propagated from a parental strain in which the expression of hydroxylase activity has remained stable.

Significance to Biomedical Research and the Program of the Institute:

The variability within a presumed homogeneous parental population is relevant to the interpretation of experiments involving somatic cell hybridization and the transformation of cells by polycyclic hydrocarbons.

Proposed Course of Project:

Further study of the differences between clones having different hydroxylase activities.

Serial No. NCI-4522

1. Chemistry Branch, Carcinogenesis, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Molecular Mechanisms of Aryl Hydrocarbon Hydroxylase (AHH) Induction in Mammalian Cell Culture

Previous Serial Number: Same

Principal Investigator: James P. Whitlock, Jr., M.D.

Other Investigator: Harry V. Gelboin, Ph.D.

Cooperating Units: None

Man Years:

Total	0.8
Professional	0.4
Other	0.4

Project Description

Objectives:

To understand the biochemical mechanisms of regulation of the AHH system in cells cloned from rat liver.

Methods Employed:

Cell culture; spectrophotofluorometry; recording spectrophotometry; agarose-polyacrylamide gel electrophoresis; radioactivity measurements by scintillation counting.

Major Findings:

The major properties of AHH in an epithelial rat liver cell strain have been described: the requirements for AHH activity, the kinetics of AHH induction; the requirements for macromolecular synthesis, the sensitivity to low level of inducers, and the mechanism of induction have been investigated.

Significance to Biomedical Research and the Program of the Institute:

AHH, a microsomal mixed-function oxygenase, is found in most tissues of many species, including humans, and plays an important role in both the detoxification and activation of carcinogenic polycyclic hydrocarbons. An understanding of the control of this enzyme complex is relevent both to chemical carcinogenesis and drug metabolism and to the problem of biochemical events in the regulation of enzyme induction.

Proposed Course of Project:

This system is useful for the study of microsomal hydroxylase, for the study of the effect of polycyclic hydrocarbons on the cell, and for the study of the molecular biology of hydroxylase induction.

Publications

Whitlock, J.P., Jr. and Gelboin, H.V.: Induction of Aryl Hydrocarbon (Benzo[a]pyrene) Hydroxylase in Liver Cell Culture by Temporary Inhibition of Protein Synthesis. J. Biol. Chem. 248:6116, 1973.

Whitlock, J.P., Jr. and Gelboin, H.V.: Aryl Hydrocarbon (Benzo[a]pyrene) Hydroxylase Induction in Rat Liver Cells in Culture. J. Biol. Chem. (in press), 1974.

Serial No. NCI-4523
1. Chemistry Branch, Carcinogenesis, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Measurement of Aryl Hydrocarbon Hydroxylase (AHH) in Human Tissues

Previous Serial Number: Same

Principal Investigator: James P. Whitlock, Jr., M.D.

Other Investigators: Harry V. Gelboin, Ph.D., Robert C. Bast, Jr., M.D.

Cooperating Units: Biology Branch, NCI

Man Years:

Total	0.2
Professional	0.1
Other	0.1

Project Description

Objectives:

Examination of human tissue for AHH activity.

Methods Employed:

Cell culture; spectrophotofluorometry, blood fractionation.

Major Findings:

AHH is present in human lymphocytes and is inducible by phytohemagglutimin and pokeweed mitogen and by the polycyclic hydrocarbon benz[a]anthracene.

Significance to Biomedical Research and the Program of the Institute:

AHH has been implicated in the detoxification and activation of chemical carcinogens; AHH has been implicated in tumorigenesis in experimental animals. An easily available human tissue with AHH activity would contribute to the study of chemical carcinogenesis and drug metabolism in humans. This would facilitate and made possible the analysis of the variability of this enzyme in humans and its role in chemical carcinogenesis in humans.

Proposed Course of Project:

Examination of peripheral leukocytes for AHH activity. Examination of human cells in culture for the metabolism of carcinogenic polycyclic hydrocarbons. Define the profile of PCH metabolism in human cells.

Serial No. NCI-4766
1. Chemistry Branch
   Carcinogenesis, DCCP
2. Molecular Carcinogenesis
   Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cell Regulatory Controls of Microsomal Hydroxylase Metabolizing Polycyclic Hydrocarbons

Previous Serial Number: Same

Principal Investigators: Friedrich J. Wiebel, M.D., Harry V. Gelboin, Ph.D.

Other Investigators: John P. Bader, Ph.D.

Cooperating Units: Cell Growth Regulation Section, CG, DCCP, NCI

Man Years:

Total:	0.6
Professional:	0.2
Other:	0.4

Project Description

Objectives:

To study the control mechanisms regulating the synthesis and degradation of the microsomal enzyme system that metabolizes polycyclic hydrocarbons in a controlled environment such as tissue culture.

Methods Employed:

Cell Culture techniques, fluorospectrophotometry, recording spectrophotometry, radioisotope techniques, and acrylamide gel electrophoresis.

Major Findings:

Induction of the enzyme system metabolizing polycyclic hydrocarbons requires de novo synthesis of RNA. To elucidate the RNA species needed for the induction process, cells in culture were used which are temperature sensitive in respect to the processing of ribosomal RNA. The results indicate that induction is independent of the formation of the larger ribosomal RNA species. The induction specific RNA apparently can be synthesized and transferred to the cytoplasm in the absence of ribosomal RNA processing.

Significance to Biomedical Research and the Program of the Institute:

The enzyme system is a key factor in the response of the organism to potentially toxic and carcinogenic foreign compounds. Understanding its mechanism of regulation will aid in its manipulation in experimental systems of chemical carcinogenesis and may ultimately help in the modification of the body's defense against chemical carcinogens.

Proposed Course of Project:

To study the mechanisms that regulate the level of enzyme activity. To devise means to control the activity of the enzyme system and the direction of alternative metabolic pathways. To determine the role of the enzyme complex in chemical carcinogenesis in vitro.

Serial No. NCI-4560
1. Chemistry Branch
   Carcinogenesis, DCCP
2. Molecular Carcinogenesis
   Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Oxidation of Carcinogenic Polycyclic Hydrocarbons in Higher Plants

Previous Serial Number: None

Principal Investigators: Friedrich J. Wiebel, M.D., Harry V. Gelboin, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.6
Professional:	0.3
Other:	0.3

Project Description

Objectives:

To determine whether plants have the capability to degrade carcinogenic polycyclic hydrocarbons. To determine whether this occurs through similar enzyme activities as in mammalian cells.

Methods Employed:

Fluorospectrophotometry, recording specrophotometry, high pressure liquid chromatography.

Major Findings:

Preparations derived from seedlings of beans and peas are capable of converting benzo[a]pyrene to hydroxylated derivatives. The reaction is dependent on the presence of protein and of molecular oxygen. It does not require NADPH and is not inhibited by the presence of carbon monoxide. Thus, benzo[a]pyrene hydroxylating activity in plants is dissimilar to hydroxylases from mammals, lower animals and bacteria. The spectrum of metabolites formed in plant material also differs from that formed in mammalian cells.

Significance to Biomedical Research and the Program of the Institute:

Polycyclic hydrocarbons are ubiquitous contaminants of the environment. Many polycyclic hydrocarbons are carcinogenic in a variety of experimental animals and probably in man. It is important to understand the dynamics of polycyclic hydrocarbon removal from the environment and particularly the possible role of the vast plant kingdom in this process. Elucidation of polycyclic hydrocarbon oxygenation in lower organisms also aids the understanding of the highly complex metabolism of these compounds in animals.

Proposed Course of Project:

To further characterize the nature of the hydroxylating activity in plants and the metabolic products.

Serial No. NCI-4779
1. Chemistry Branch
Carcinogenesis, DCCP
2. Molecular Carcinogenesis
Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Evaluation of Various Compounds for Their Effects as Inducers and Inhibitors of Aryl Hydrocarbon Hydroxylase Activity and Tumorigenesis

Previous Serial Number: Same

Principal Investigator: Friedrich J. Wiebel, M.D.

Other Investigators: Harry V. Gelboin, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.6
Professional:	0.8
Other:	0.8

Project Description

Objectives:

To develop methods to determine the activity of various compounds as inducers or inhibitors of carcinogen hydroxylating enzymes and polycyclic hydrocarbon tumorigenesis.

Methods Employed:

Cell culture techniques, spectrophotofluorometry, recording spectrophotometry, fluorescent microscopy and radioisotope studies and mouse skin tumorigenesis.

Major Findings:

Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), two antioxidants used as preservatives in human and animal food, have been investigated as inducers and inhibitors of the microsomal mixed-function oxidases involved in the metabolism of polycyclic hydrocarbons. Both compounds are potent inhibitors in vitro of the hepatic enzyme from untreated rats and have no or only weak inhibitory effects on the enzyme from polycyclic hydrocarbon treated animals. Application of BHT with food to rats increases hepatic aryl hydrocarbon hydroxylase activity more than two fold within one week, and decreases the enzyme activity in lung by 50%. The

results indicate that the antitumorigenic effect of the antioxidants may be related to their effect on the enzyme system metabolizing carcinogenic polycyclic hydrocarbons.

Significance to Biomedical Research and the Program of the Institute:

Compounds of the type investigated can be used to explore the mechanism of enzymatic activation of carcinogenic polycyclic hydrocarbons and of tumor initiation by chemical carcinogens. It also is important to understand the influence of these food additives on the enzyme systems metabolizing xenobiotics and consequently on their biological effects.

Proposed Course of Project:

To use antioxidants such as BHA or BHT to explore the activation mechanism of carcinogenic polycyclic hydrocarbons, to alter the course of tumorigenesis.

Honors and Awards

Presentation of "Enzyme induction and metabolism of polycyclic hydrocarbons in cell culture, experimental animals and man." Workshop on Approaches to Assess the Significance of Experimental Chemical Carcinogenesis Data for Man, Brussels, Belgium, December 10-12, 1973.

Presentation of "Mammalian mixed-function oxidases: Mechanism of induction and role in carcinogenesis." A symposium organized by the American Society of Zoologists, Houston, Texas, December 28-30, 1973.

Publications

Wiebel, F.J. and Gelboin, H.V.: Enzyme Induction and Polycyclic Hydrocarbon Metabolism in Cell Culture, Experimental Animals and Man. IARC Monograph Volume 4, 1974 (in press)

Serial No. NCI-4529
1. Chemistry Branch, Carcinogenesis, DCCP
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: DNA Replication in Prokaryotic and Eukaryotic Cells

Previous Serial Number: Same

Principal Investigators: C. Wesley Dingman, M.D. and Minoru Ishizawa, Ph.D.

Other Investigators: Tsuyoshi Kakefuda, M.D., Ph.D., Tina Bak and Sylvia Bunting

Cooperating Units: Protein Section, Chemistry Branch

Man Years:

Total	1.3
Professional	1.2
Other	0.1

Project Description

Objectives:

To learn more about the molecular mechanisms of DNA synthesis, in particular the role of short, nascent DNA fragments and the role of the secondary and tertiary structure of DNA in the region of the replication point.

Methods Employed:

This project was initiated using E. coli as the organism to be studied. Much more is known regarding the mechanisms of DNA synthesis in this organism, and the major problems left unanswered are best delineated with these organisms. If we are successful in advancing our knowledge regarding DNA synthesis in this system, we intend to apply the techniques and knowledge we have gained to studies of DNA synthesis in human cells in culture.

We are using both thymine requiring wild-type cells and those with mutations affecting DNA replication, DNA repair, and DNA recombination. Our chief technique is to prelabel parental DNA by overnight labeling with ^{14}C-thymine and then pulse label newly synthesized DNA with ^{3}H-thymidine for 5 to 180 sec at 20°C. The DNA is then isolated and the size distribution and labeling characteristics of the nascent, newly-synthesized, DNA fragments examined, usually by polyacrylamide gel electrophoresis. The molecular conformation of

the replication point is examined by means of its electrophoretic mobility in polyacrylamide gels before and after heat denaturation and before and after treatment with various specific nucleases. Zone sedimentation analysis in alkaline and neutral sucrose gradients is also employed. Results obtained with in vitro DNA synthesis using highly purified preparations of E. coli polymerase I are compared with those obtained in vivo.

Major Findings:

In confirmation of the results obtained by others studying DNA replication in bacteriophages, we find strong evidence for the existence of single-stranded regions in the neighborhood of the replication point in E. coli. We have found that polyacrylamide gel electrophoresis allows us to separate forked replication points, high molecular weight DNA containing single-stranded regions, high molecular weight double-stranded DNA, and the various size classes of low molecular weight single-stranded DNA fragments, each from the other.

Our results have indicated that the number of independent DNA polymerizing regions per fork (which is one more than the number of nascent, low molecular weight, precursor fragments per fork in the Okazaki model of DNA replication) is dependent upon the growth conditions in the bacterial culture. Thymine starvation and lower temperatures (20°) favor the existence of many polymerizing regions per fork (8 to 10) while adequate thymine concentrations in the external medium and higher temperatures (37°) favor the existence of only two polymerizing regions per fork.

Significance to Biomedical Research and the Program of the Institute:

The replication and repair of DNA are critical and vital steps in the economy and survival of all cells. The mechanisms by which they are accomplished have not yet been elucidated nor has the role that these processes play in malignant transformation been discovered, in spite of widespread belief that the regulation of DNA replication may be an important key to understanding malignancy. This project is an attempt to gain some further insights into the process of DNA replication, first in prokaryotes and later in eukaryotes. Understanding even prokaryotic DNA replication should be of value in studies on the mechanism of malignant transformation of eukaryotic cells by oncogenic viruses.

Proposed Course of Project:

To pursue the goals as outlined above in Objectives.

Publications

Dingman, C.W.: Bidirectional Chromosome Replication: Some Topological Considerations. J. Theoret. Biol. (In Press).

Dingman, C.W., Fisher, M.P., and Ishizawa, M.: DNA Replication in E. coli: Physical and Kinetic Studies of the Replication Fork. J. Mol. Biol. (In Press).

Serial No. NCI-4787
1. Chemistry Branch,
Carcinogenesis, DCCP
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Mode of Replication of RNA Tumor Viruses

Previous Serial Number: Same

Principal Investigator: Tsuyoshi Kakefuda, M.D., Ph.D.

Other Investigators: C.W. Dingman, M.D., and Tina M. Bak

Cooperating Units: None

Man Years:

Total	:	1.0
Professional	:	0.5
Other	:	0.5

Project Description

Objectives:

To observe the early events of gene replication of RNA tumor viruses in host cells.

Methods Employed:

(1) Electron microscopic autoradiography of ^{3}H-thymidine labeled 3T3/BALB and chick embryo cells infected with murine sarcoma virus or Rous sarcoma virus was performed in order to detect DNA transcription of viral RNA at different post-infection times. (2) Subcellular organelles were isolated from virus infected cells and the endogenous *in vitro* activity for DNA synthesis was measured. The DNA so synthesized was denatured in alkali, hybridized with high molecular weight viral RNA and analyzed by Cs_2SO_4 isopycnic centrifugation in order to determine the sequence homology to the viral RNA. (3) The nucleic acids extracted from the isolated subcellular organelles from virus infected cells (^{3}H-TdR labeled) were hybridized with high molecular weight viral RNA. Fractions containing hybrid molecules were collected after centrifugation in Cs_2SO_4 and denatured in alkali. The virus specific DNA thus isolated was analyzed in alkaline Cs_2SO_4, alkaline sucrose gradient centrifugation or re-hybridized with the viral RNA.

Major Findings:

Incorporation of ^{3}H-TdR was observed in the region of the plasma membrane beginning one hour after virus infection by electron microscopic autoradiography. The photopositive grains dispersed more diffusely in the cytoplasm as the post-infection time was prolonged from 2.5 to 24 hours. Such ^{3}H-TdR incorporation was not observed in uninfected cells. Synthesis of DNA in vitro, using subcellular organelles from infected cells, was measured and found to be especially active when the plasma membrane fraction was used. The DNA synthesized in this manner was specifically hybridizable with viral RNA. The molecular weight was estimated as 1.5 x 10^{5} daltons and homogeneous in size. The DNA synthesized in the virus infected cells, isolated and analyzed by the methods described above, indicated that the initial transcription of viral RNA to DNA occurs one hour after infection at the plasma membrane. The virus specific DNA is heterogeneous in size ranging from 10^{5} to higher than 10^{6} daltons and may contain a complete copy of the viral RNA.

Significance to Biomedical Research and the Program of the Institute:

The transcription of viral RNA to DNA and the incorporation of the latter into the genetic system of the host cell are the important steps in viral carcinogenesis. The mechanism of viral replication and the nature of the intermediate DNA products in the cell are, however, largely unknown. The results obtained from the three different experiments described above provide useful information on the sequence of events occurring in virus infected cells, particularly with respect to the role of the plasma membrane. This is of particular interest since some modification of this membrane by drugs or immunological treatment might offer a means for aborting malignant transformation of cells by RNA tumor viruses.

Proposed Course of Project:

Enucleated cells prepared by cytochalasin B treatment are to be challenged by RNA tumor viruses. Virus specific DNA accumulated in the cytoplasm will be isolated and characterized by biochemical and biophysical methods. Cross hybridization experiments with the viral DNA (synthesized in vitro and in vivo), high molecular weight viral RNA, and chromosomal DNAs of normal and transformed cells, will be carried out for the further analysis of the integration of the viral genome in host cells. Hybrid molecules will be studied by electron microscopy using the protein monolayer technique, and physical mapping of the molecules will be attempted in order to locate the region of genome responsible for transformation.

Publications

Kakefuda, T., Dingman, C.W., Bak, T.M., Hatanaka, M., and Kitano, Y.: Reverse Transcription of Viral Genome Associated with Plasma Membrane after Infection with RNA Tumor Viruses. Cancer Res. (In Press).

Serial No. NCI-4785
1. Chemistry Branch, Carcinogenesis, DCCP
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Role of DNA Repair Mechanisms in the Etiology of Cancer

Previous Serial Number: Same

Principal Investigators: Rufus Day, III, Ph.D. and C. Wesley Dingman, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total	:	1.0
Professional	:	1.0
Other	:	0.0

Project Description

Objectives:

To learn more about DNA repair mechanisms in mammalian cells and about their role in carcinogenesis. In particular, to determine the nature of the biochemical defects in human cell lines grown from biopsies taken from persons having extreme susceptibility to skin carcinogenesis. The majority of such people, whose genetically inherited malady is termed Xeroderma Pigmentosum (XP), have defects in the repair of ultraviolet damage to DNA. A particularly interesting patient we have been concerned with has been diagnosed as having XP but his cells show completely normal DNA repair as judged by the criteria of several other investigators.

Methods Employed:

We have published a method permitting the visualization of growth of individual adenovirus particles on human fibroblast cells (our method also works for mouse adenovirus infecting mouse cells and human adenovirus infecting monkey cells). Using this method, we have been able to quantitate the deleterious effects of ultraviolet light (UV) on the ability of the virus to initiate and sustain infection. The method involves establishing a monolayer cell culture which is then infected with Adenovirus which has been irradiated with different doses of UV. The infected cells are then incubated for 17-19 days with feeding by

means of periodic overlaying with nutrient agar. Viable virions or those irradiated ones which have been "reactivated" by host cell repair mechanisms form plaques of dead, lysed cells and their number can be counted. With this method the plating efficiency using unirradiated Adenovirus is the same on all of the (non-transformed) human cell lines tested so far.

Major Findings:

(1) Virus damaged by ultraviolet light: Using the method described above we have assayed 10 normal human fibroblast cell strains, 12 XP cell strains of the repairless type (and belonging to four gene complementation groups) the 1 XP cell strains of the "normal" repair type, 2 strains of cells from patients with Fanconi's anaemia, and 1 strain of cells from a patient with Blooms syndrome, with regard to the amount of UV required to inactivate Adenovirus 2 to a given survival level, using these cells as viral hosts. We found that the virus must be irradiated with an average of 220 ± 20 Joules/meter^2 of 254nm light to obtain 37% survival measured on normal cell strains, while the following amounts are required when the XP strains are used as hosts:

XP Cell Strain	Gene Complementation Group	Dose resulting in 37% survival JOULES/meter^2
1201	A	16
XPA	A	10
1203	A	8
1223	A	7.5
1199	B	25
1161	C	74
1166	C	67
1170	C	31
1204	C	25
1160	D	8
1157	D	7.4
1200	D	7.4
XW 1162	"normal" repair	153
(10 normals		220 ± 20)

All the XP strains of the repairless type (especially group A and D strains) show a decrease (with respect to normal cells) in the amount of UV light required for 37% survival - in the most severe cases about a 30-fold decrease. The XP strain with "normal" repair appears to show a partial (30%) repair defect by our method. This technique of estimating repair is so sensitive that in experiments with 2 XP cell populations (of different complementation groups) fused together by inactivated Sendai virus, the number of heterokaryons (now capable of repair since complementation has occurred) is easily detectible.

(Experiments done in collaboration with Kenneth Kraemer, M.D., Dermatology, NCI.) The Fanconi's anaemia and Blooms syndrome strains show normal repair using the virus technique.

(2) Virus damaged by other agents: In addition to ultraviolet light, we have also used the virus assay to test for repair of damage due to nitrous acid, psoralen plus 365nm light, 3-hydroxy-benzo[a]pyrene in the presence of cupric ion, and ^{60}Co gamma rays. Damage due to treatments of the first three types is at least partly reactivated as evidenced by the finding that damaged virions show less plaque forming ability (relative to non-treated control virus) using XP cells than when using normal cells as hosts. No differences between XP (group A) cells and normal cells were found with respect to their ability to repair Adenovirus 2 damaged in air-saturated, frozen suspensions by Cobalt-60 gamma rays.

Significance to Biomedical Research and the Program of the Institute:

An evaluation of the role of DNA repair and/or related mechanisms in conferring resistance or susceptibility to mutagenesis and carcinogenesis is an important facet in any overall program having as its goal the understanding of the molecular pathways which, when perturbed, give rise to carcinogenesis. Physical, chemical, and viral carcinogens are all known to alter the structural integrity of the cellular genetic apparatus. It is a long range goal of this project to determine whether or not the elucidation of genetic repair mechanisms is important to the understanding of carcinogenesis, but it is expected that an understanding of genetic repair mechanisms in general will benefit many areas of biomedical research.

Proposed Course of Project:

To pursue the goals outlined above in Objectives and publish these results.

Publications

Day, R. S., III.: Cellular Reactivation of Ultraviolet-Irradiated Human Adenovirus 2 in Normal and Xeroderma Pigmentosum Fibroblasts. Photochemistry and Photobiology 19: 9-15, 1974.

Serial No. NCI-4558
1. Chemistry Branch, Carcinogenesis, DCCP
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Role of DNA Repair Mechanisms in In Vitro Chemical Transformation

Previous Serial Number: None

Principal Investigators: Takeo Kakunaga, Ph.D., Rufus S. Day, III, Ph.D., and C. Wesley Dingman, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total	:	1.1
Professional	:	1.1
Other	:	0.0

Project Description

Objectives:

To learn more about the mechanisms involved in the malignant transformation of cells. In particular, to attempt to develop an experimental system in which one can effect the in vitro transformation of human cell lines with chemical and/or physical carcinogens.

Methods Employed:

On the assumption that more than one (mutagenic ?) step may be involved in the malignant transformation of cells, we intend to test human cell strains established from biopsies taken from persons who have presumably inherited one such step or who have a condition known to predispose them to malignancies. These conditions include retinoblastoma, Wilms' tumor, neuroblastoma, polyposis of the colon, Xeroderma pigmentosum, Fanconi's anaemia, Bloom's syndrome, and Ataxia telangiectasia. In previous work, Dr. Kakunaga has developed the clone A321-4NQO in vitro transformation system (clone A321 is a derivative cell line from a BALB/3T3 mouse cell line). This latter system is highly dependent upon various experimental factors including choice of serum, cell density, and cell division fixation of the transformed state. Benzo[a]pyrene and 3-methyl-cholanthrene are also active in this system. We will use this mouse cell system

in parallel with experiments on human cell lines to aid us in establishing potimum cell culture conditions for transformation. Transformed human cells will be tested for malignancy by injection into immunosuppressed mice. We will also use this mouse cell system to explore the role of DNA repair mechanisms in malignant transformation; Studies which will then be extended to the human cell system when it has been developed. We are currently working to form established human cell lines from cells taken from patients having some of the conditions listed above.

Major Findings:

This project has just gotten started (January 1974) and no major findings have developed as yet.

Significance to Biomedical Research and the Program of the Institute:

To date there has been no reproducible system established in which human cells are transformed in vitro by chemicals. We believe our rationale for the establishment of such a system is as sound as any at this time. If this project is successful, it will lead the way to many experiments in chemical and physical carcinogenesis which at present can only be carried out with animal cells. It would also provide a system in which to assay potential carcinogens directly on human cells, thus removing the necessity of extrapolating from data obtained from animal cells.

Proposed Course of Project:

To pursue the goals as outlined above in Objectives and to publish any results so obtained.

Serial No. NCI-4525
1. Chemistry Branch,
Carcinogenesis, DCCP
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on Electrophoretic Techniques for Protein, RNA, and DNA

Previous Serial Number: Same

Principal Investigator: Andrew C. Peacock, Ph.D.

Other Investigators: Sylvia L. Bunting, M.S. and Roswell A. Reed

Cooperating Unit: None

Man Years:
Total: 2.8
Professional: 2.8
Other: 0.0

Project Description

Objectives:

To devise analytical approaches for the studies of synthesis of protein, RNA, and DNA and to use these techniques to gain an understanding of control processes involved in the synthesis of these compounds.

Methods Employed:

Cultures of HeLa cells, assay of radioisotopes, isolation and electrophoresis of RNA and DNA, analysis of nucleotides and nucleosides by chromatography, optical scanning of stained and unstained gels.

Major Findings:

1. Prolactin doubles the rate of uridine permeation in mammary cells. That prolactin increases the rate of incorporation of uridine into RNA was reported previously. The rate of permeation is an important variable in this process. Cells were incubated with various amounts of uridine with and without prolactin and the rate of permeation measured. At levels of uridine less than 10^{-4} M the rate of permeation was doubled after prolactin treatment. At high concentrations of uridine

the effect was less marked. We could not tell from our data which of the kinetic parameters (Km or Vmax) was primarily affected because of experimental difficulties in determining rates at very low uridine concentrations.

2. Salt concentration used in extraction of RNA affects molecular state of RNA. High molecular weight RNA extracted from cells in the presence of 0.5 M sodium chloride gave rise to a high molecular weight fraction. This material was isolated from the gel by treatment with phenol and buffered SDS and some of its properties were studied. There was no evidence of unusual resistance to ribonuclease, thus ruling out some unusual double-stranded configurations that may have given rise to the aggregate. Melting experiments were conducted on the purified material which revealed contamination with nucleases. The nuclease contamination was taken as evidence for the presence of protein contaminants even though treatment with proteolytic enzymes was without effect. Treatment of the aggregate with DMSO revealed that the RNA constituent was exclusively HnRNA. The extraction procedure and subsequent DMSO treatment thus provide a somewhat inconvenient means for the isolation of HnRNA and, on the other hand, a means of preparing ribosomal proteins free of HnRNA.

3. Studies on the electrophoretic properties of 18S rRNA. The 18S rRNA of HeLa is found on high resolution electrophoresis as three similar but distinct bands. Investigations have been undertaken to find out whether these are conformational isomers of the same polynucleotide chain or are distinct with respect to sequence or length. Melting experiments have shown that two of the forms may be converted to the third suggesting that they are isomers. Attempts are being made to delineate the <u>in vitro</u> conditions which lead to interconversion.

4. Electrophoretic properties of transfer RNAs. The mobility of tRNAs in gel electrophoresis has been found to be a function of salt concentration. The fastest mobilities at 20° are found at 0.1 M KCl. These changes are consistent with the literature on conformational changes in tRNA as a function of salt concentration and indicate that tRNA is most rapidly migrating when in its most compact structure. The inclusion of positively charged groups in the gel matrix has a profound effect on the electrophoretic properties of tRNA. Dependent upon the salt concentration the mobilities are much reduced presumably as a result of ion exchange phenomena that occur with the positively charged groups in the gel. Under these circumstances the most rapidly migrating tRNAs become the slowest. The possibility of fractionation of tRNA utilizing this phenomenon is being explored.

5. Gel electrophoresis affects separation of left and right strands from denatured mouse satellite DNA. The two bands previously found to result from the denaturation of mouse satellite DNA were identified by two means:

a) The dense strand contains two times more thymidine than the lighter band. Electrophoresis of thymidine-labeled mouse satellite DNA showed that the specific activity of the slower migrating band was twice that of the faster migrating band, thereby identifying the slower migrating band with the dense band found on ultracentrifugation.

b) Smaller quantities of dense and light bands were isolated by isopycnic centrifugation and found to migrate as the slower and the faster band, respectively. This result was in contrast to our previous studies showing that dense double-stranded DNAs migrate more rapidly.

Significance to Biomedical Research and the Program of the Institute:

The ability to conduct detailed studies on interrelationships between macromolecules, such as RNA, proteins, and DNA, is probably fundamental to understanding the alterations which occur in malignancy. The above studies are directed towards this goal.

Proposed Course of Project:

Most of the studies described above are currently incomplete, and will be continued. The mechanisms by which DNA synthesis is controlled will be studied by a comparison between experimental results and computer simulation of proposed models.

Serial No. NCI-4562
1. Chemistry Branch,
Carcinogenesis, DCCP
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on Properties of Cytoplasmic RNAs in Mammalian Cells

Previous Serial Number: None

Principal Investigator: Keiji Nishinaga, Ph.D.

Other Investigators: Andrew C. Peacock, Ph.D. and Sylvia L. Bunting, M.S.

Cooperating Unit: None

Man Years:

Total:	1.2
Professional:	1.2
Other:	0.0

Project Description

Objectives:

To devise analytical approaches for studies of heterogeneity of 18S rRNAs and to use these techniques to get an understanding of control process of RNA synthesis in mammalian cells.

Methods Employed:

Cultures of HeLa cells and mouse L cells, assay by radioisotopes and sucrose gradient centrifugation, electrophoresis and optical scanning of stained gels.

Major Findings:

1. Analysis of 18S RNA in rat liver by electrophoresis. Electrophoresis of 18S RNA in 2.2% or 2.5% acrylamide-0.5% agarose gels gave some indication that 18S RNA isolated by a cold extraction has at least two bands whereas these same RNAs isolated with the hot extraction show another two bands.

The electrophoresis of 18S RNA, isolated by cold phenol extraction, treated by 6 M urea showed three bands. One of these three bands is in the area between the two bands of 18S

RNA originally present. With labeled 18S RNA similar observations were made on RNA extracted with hot phenol.

2. Species specificity. Double bands of 18S RNA were seen in HeLa cells and mouse liver RNAs. 28S RNA isolated by cold extraction from a number of different animal cells (HeLa cells, Chinese hamster fibroblasts, chick embryo fibroblasts, and Novikoff rat hepatoma cells) is a hydrogen-bonded complex between 28S rRNA and a small molecule (7S RNA) of about 130 nucleotides. At present, we cannot conclude whether these double bands of 18S RNA are due to conformational change, if they are different species of RNA, or if they, like 28S RNA, contain additional small RNA. However, if these RNAs are shown to be different species, I will try to study another aspect of this work: their functional specificity and cell specificity using both normal and tumor cells.

Significance to Biomedical Research and the Program of the Institute:

The ability to conduct detailed studies on interrelationships between RNA and DNA is probably fundamental to understanding the control mechanism which differentiates between normal and malignancy. The above studies are directed towards this goal.

Proposed Course of Project:

Most of the studies described above are currently incomplete and will be continued.

Serial No. NCI-4563
1. Chemistry Branch,
Carcinogenesis, DCCP
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies of a Cationic Carbocyanine Dye

Previous Serial Number: None

Principal Investigator: Marie R. Green, Ph.D.

Other Investigators: Julia V. Pastewka

Cooperating Unit: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

To obtain more information about the characteristics of the dye 1-ethyl-2-[3-(1-ethylnaphtho[1,2d] thiazolin-2-ylidene)-2-methyl-propenyl] -naphtho[1,2d] thiazolium bromide and its interaction with macromolecules.

Methods Employed:

Absorption measurements of dye solutions in the Cary 15 recording spectrophotometer, scanning of gels in a Gilford spectrophotometer, observations of color reactions with films of pure substances and tissue sections with a microscope.

Major Findings:

A method for staining phosphoproteins on gels and identifying phosphoproteins histochemically in tissue sections was developed using this dye. In the course of the latter investigation it was necessary to survey many tissues to demonstrate that phosphoproteins could be distinguished from the staining of other tissue constituents. It was found that under appropriate conditions other classes of macromolecules stained differentially. The dye stained nuclei purple, mast cells red purple, proteins pink or red, cartilage blue-purple and mucins

blue-green. The nature of the macromolecules involved in the various color reactions was ascertained by the use of films of pure substances and by various chemical and enzymatic treatment of tissue sections. It was possible to distinguish proteoglycnans from glycoproteins by color.

Significance to Biomedical Research and the Program of the Institute:

This newly developed staining procedure is simple, requires one hour staining and specifies classes of macromolecules by color. It should find wide application as a quick method for the identification of secretions and cellular inclusions in pathologic diagnosis and in research investigations.

Proposed Course of Project:

Studies of the dye interaction with glycoproteins on acrylamide gels are in progress. Application of the histochemical method will be made utilizing the differential properties of the dye to specific problems in pathology at the chemical and tissue level. Biopsy material will be taken to assess what advantages this dye may have over other methods in current use.

Publications

Green, M. R. and Pastewka, J. V.: Simultaneous differential staining by a cationic carbocyanine dye of nucleic acids, proteins and conjugated proteins. II. Carbohydrate and sulphated carbohydrate containing proteins. J. Histochem. Cytochem. (In Press).

Serial No. NCI-4782
1. Chemistry Branch,
Carcinogenesis, DCCP
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Hormonal Effects on Normal and Malignant Breast Tissue in Culture

Previous Serial Number: Same

Principal Investigator: Marie R. Green, Ph.D.

Other Investigators: Julia V. Pastewka and Andrew C. Peacock, Ph.D.

Cooperating Unit: None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

To study the response of normal and malignant breast tissue in culture to hormones in terms of RNA and DNA synthesis and the production of proteins (particularly milk proteins).

Methods Employed:

Culture of mammary explants, isolation of RNA and DNA, separation on agarose-acrylamide gels, radioactive tracer techniques, isolation and purification of proteins from explants and milk, polyacrylamide gel electrophoresis, examination of tissue sections by histochemical methods, autoradiography.

Major Findings:

We have identified some of the proteins made by mammary explants in organ culture. Proteins of explants from virgin, mid-pregnant, late-pregnant mice and from explants of malignant breast tumor tissue have been separated by gel electrophoresis. These have been identified by their relative migration with respect to a marker, by staining techniques for glycoproteins and phosphoproteins and by incorporation of radioisotopes.

Double isotope labeling techniques have increased the sensitivity of the previously described bioassay for prolactin response.

A method for identifying phosphoproteins on acrylamide gels was developed using the cationic carbocyanine dye 1-ethyl-2-[3-(1-ethylnaphtho[1,2d]thiazolin-2-ylidene)-2-methylpropenyl]-naphtho-[1,2d]thiazolium bromide. This dye was previously used as a sensitive indicator for nucleic acids. Several proteins were analyzed for phosphorus content. The caseins (α and β) and phosvitin stained blue on gels, but following the removal of phosphate groups with alkaline phosphatase the proteins stained red. This method was used to characterize the caseins in mouse milk and mammary explants.

No adequate histochemical method was available for phosphoproteins. It seemed possible that this dye might be especially useful for such purposes. The method for staining gels was not suitable. Following extensive testing of buffers, hydrogen ion concentration and ionic strength a method for the identification of phosphoproteins in tissue sections was developed. This method is being used to identify phosphoproteins in secretions of mammary gland.

Significance to Biomedical Research and the Program of the Institute:

Hormone response can be measured on small pieces of mammary tissue in culture. The methods developed in the mouse system may be used on biopsy material from hosts (animal or human) bearing malignant tumors. Testing of biopsy material may aid evaluations on whether specific ablative or drug therapies are indicated.

The method for identifying phosphoproteins on gels and sections will find wide application in many areas of investigations which involve the study of such proteins. We will find it valuable in our future studies of milk and mammary gland.

Proposed Course of Project:

The study of the mechanism of hormone regulation in normal and malignant mammary tissue will continue.

Publications

Green, M. R., Pastewka, J. V. and Peacock, A. C.: Differential staining of phosphoproteins on polyacrylamide gels with a cationic carbocyanine dye. Anal. Biochem. 56: 43-51, 1973.

Green, M. R. and Pastewka, J. V.: Simultaneous differential staining by a cationic carbocyanine dye of nucleic acids, proteins, and conjugated proteins. I. Phosphoproteins. J. Histochem. Cytochem. (In Press).

SUMMARY REPORT

EXPERIMENTAL PATHOLOGY BRANCH

July 1, 1973 through June 30, 1974

"Plans, develops, and implements a coordinated research program involving (1) development and characterization of experimental models of major forms of human cancer; (2) development of techniques for experimental study of the etiology and pathogenesis of cancer in human tissues; (3) analysis of stages in pathogenesis of human cancer and their suitability for the development of preventive measures; (4) biochemical studies on the metabolism of chemical carcinogens in selected animal tissues; and (5) development of standards of pathology for the evaluation of carcinogenic effects."

Within this Branch the induction of neoplastic disease is studied at all levels of biological organization, from intracellular macromolecules to intact animals. These studies include covalent binding of chemical carcinogens to nucleic acids; repair phenomena which modify the consequences of such binding; the effects of exogenous substances such as hormones, chalones, and vitamin A on differentiation and multiplication of carcinogen-treated cells; the pharmacodynamics of carcinogen transport and distribution which tends to localize these compounds or their metabolites in specific target tissues; and analysis of genetic factors contributing to the inducibility of specific forms of cancer among individuals of a given species.

The former Carcinogen Screening Section of this Branch was separated in April 1973 to become the nucleus of the newly created Carcinogen Metabolism and Toxicology Branch. The Experimental Pathology Branch consists of five research groups: Digestive System Carcinogenesis Section, Endocrine Carcinogenesis Section, In Vitro Pathogenesis Section, Perinatal Carcinogenesis Section, and Ultrastructure Unit. It also contains a Histopathology Unit, which provides histology service to the Carcinogenesis Program laboratories. By the end of this fiscal year, most of the Endocrine Carcinogenesis Section will be relocated at the NCI's Frederick Cancer Research Center, Ft. Detrick, Maryland. The staff of this Branch is largely involved in the scientific development and monitoring of the Biological Models Segment. Members of the staff also served until recently as project officers for contracts in other segments.

Digestive System Carcinogenesis Section *"(1) Plans and conducts research on the etiology and pathogenesis of cancer of the human digestive system including pancreas, liver, esophagus, stomach, colon and rectum. (2) Develops experimental models for laboratory investigation of the induction and development of those forms of cancer. (3) Utilizes experimental models to develop techniques for prevention of cancer of the digestive system in humans by interrupting the initial response to chemical and physical carcinogens or by arresting or reversing subsequent progression of the disease process."*

Dr. Richard S. Yamamoto, a member of this Section, spent 13 months at the Japanese National Cancer Center Research Institute and at the Institute of

Medical Sciences, University of Tokyo, in Tokyo, Japan, completing studies on carcinogenesis of the stomach and colon. Though its death rate is declining in the United States, stomach cancer still annually claims an estimated 14,000 lives. In Japan, stomach cancer is the major form of cancer with a death rate 7 times as high. On the other hand, the incidence of cancer of the colon and rectum in the United States is very high and has plateaued at a rate 2.2 times that found in Japan, where the incidence has risen continuously for the past 15 years. The following studies were done in collaboration with Doctors T. Sugimura, T. Matsushima, and T. Kawachi of the above Institutes. (1) The effect of high sodium chloride intake on the induction of glandular stomach tumors in mice by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). The results indicate that simultaneous feeding of high sodium chloride and administration of MNNG was not effective, but subsequent feeding of high salt after MNNG did increase the incidence and the severity of cancers of the glandular stomach. (2) Role of protease on carcinogenesis of colon and stomach, (a) A protease inhibitor, leupeptin, isolated from Actinomycetes, decreased the incidence but did not inhibit the growth of tumors in the colon induced by azoxymethane; (b) The effect of leupeptin on gastric carcinogenesis by a direct acting carcinogen, MNNG, was also investigated. This experiment is still in progress. (3) A new area of study was undertaken on the role of protease in development of metastasis.

Another research project within the Section concerns the investigation of repair of carcinogen-induced damage to DNA in both human and rodent cells. Repair processes subsequent to damage with either N-acetoxy-2-acetylaminofluorene or ultraviolet light are distributed approximately equally in both satellite and mainband DNA, and are present in human cells in DNAs of all degrees of kinetic complexity. Thermal elution profiles of repaired sections of DNA are compatible with the hypothesis that bases inserted during repair synthesis are hydrogen bonded to the template strand and represent a stable and therefore probably an accurate copy of it. These data indicate that repair synthesis in mammalian cells is at least not grossly inaccurate. Future studies on the significance of repair phenomena in the process of carcinogenesis will focus on agents which enhance or inhibit repair synthesis.

At the conclusion of this fiscal year, the DNA repair program will be transferred from this Section and consolidated with the related programs of the In Vitro Pathogenesis Section.

Endocrine Carcinogenesis Section *"(1) Develops experimental models of cancer of endocrine target organs including prostate, breast, uterus and ovary. (2) Utilizes these models to conduct investigations on conditions affecting the sensitivity of endocrine target organs to endogenous and exogenous chemical and physical carcinogens. (3) Studies the etiology and pathogenesis of human cancer of endocrine target organs to develop measures which can be used to prevent the induction or development of the disease."*

Prostate Carcinogenesis: A major study of the normal histological and morphological development of the mouse and rat prostate has been completed. This study has focused on the comparative development of the ventral,

dorsal, and lateral lobes of the prostate at prenatal, neonatal, prepubertal, young adult, and old age stages. Comparisons of secretory epithelial cell height and acinar structure have been made. A reliable classification of lobular differentiation using epithelial cell height, stromal density, number of muscular fibers, and acinar shape has evolved from these studies. In addition, differences in the rate of lobular development have been found. These findings have been correlated with an electron microscopic study of prostatic epithelium.

Using the information gained from the histological study, efforts to induce prostatic adenocarcinoma using 7,12-dimethylbenz[a]anthracene (DMBA), methylcholanthrene, and ethylnitrosourea (ENU) have been continued. These studies have incorporated alterations induced by endocrine imbalance as well as old age. Adenocarcinoma induction has been accomplished with ENU in rats.

Prostate transplanted to the cleared mammary fat pad of an androgenized female rat has survived for more than 18 months. This transplant site has allowed repeated carcinogen treatments and has provided an easily observable area to assess prostatic transformation. This transplant site has also been used for conditioning prior to organ culture. Prostate grown in this area in an androgenized female shows increased numbers of epithelial cells without a proportional increase in stromal elements. The conditioned prostate can be more easily maintained in organ culture, and yields a greater ratio of epithelial cells to fibroblasts for cell culture.

Mammary Carcinogenesis: An examination of the influence of age on the induction of mammary adenocarcinoma has revealed a direct correlation of the amount of DMBA bound to mammary DNA with the incidence of tumors. Rats fed at 50 days of age have a 100% incidence of mammary cancer. If DMBA is administered at 35 days of age, tumor incidence is approximately 50-60%. Old animals (120 days old) have a much reduced incidence (10%). In animals fed DMBA at 35 or 120 days of age, the binding of DMBA to DNA was not detectable as early as that in 50 day old rats and binding did not persist. The depression in binding was closely related to the depression in tumor incidence. The rat liver at these ages is refractory to DMBA tumorigenesis, and no correlation between age, tumor incidence or binding of DMBA to liver macromolecules could be found. Other studies indicated that the sensitivity of the mammary epithelium to estrogen, progesterone, and prolactin changes with age. These hormones elicited a greater degree of DNA synthesis in older than in younger animals.

A method was developed to grow normal human breast tissue in cell culture, using techniques developed for the separation and isolation of rat mammary epithelial cells free from contaiminating fibroblasts. Hormonal requirements both quantitative and qualitative have been established. This system is now being used to examine hormone and carcinogen action on human breast epithelial cells in culture. It is hoped that this system will serve as a point of correlation between human and animal studies on the mechanism of action of hormones and carcinogens.

In Vitro Pathogenesis Section *"(1) In collaboration with other sections of the Branch, develops in vitro models for studying the pathogenesis of major forms of human cancer utilizing both human and animal tissues. (2) Utilizes these culture systems in biochemical, cytologic and biologic investigations to develop approaches to the prevention of cancer in humans. Stages in the pathogenesis of cancer are studied to learn which are amenable to arresting or reversing the progression of the disease before it threatens life. (3) Develops procedures for evaluating the biologic potentiality of lesions induced by chemical or physical carcinogens in human tissues in vitro."*

This Section has directed its efforts at developing in vitro model systems for use in a mechanistic approach to carcinogenesis. Much of this effort has been directed at developing a system of epidermal cell culture to use as an in vitro parallel of the in vivo skin carcinogenesis system, which is a convenient experimental system for the study of squamous-cell carcinomas. This laboratory has been able to isolate and grow in culture pure epidermal cells from newborn mice as well as pure populations of hair follicle cells and dermal fibroblasts. The epithelial cells keratinize in vitro and, when grafted back to prepared skin graft beds of syngeneic hosts, produce intact donor skin. These cells can be transformed in vitro by chemical carcinogens. In addition, these cells can be altered by treating the newborn donors transplacentally with urethane several days prior to delivery. These cells also respond to the promoting agent 12-O-tetradecanoyl-phorbol-13-acetate (TPA) by a marked hyperplastic response in vitro, similar to that observed in vivo. This method offers an opportunity to study promotion as well as differentiation in vitro.

A major effort has been directed at understanding the differentiation of epidermal cells and the role this process plays in malignant transformation. To this end epidermal cell differentiation has been successfully altered in vitro by the use of retinyl acetate. The cells change from a keratinizing to a secretory epithelium. There is a two-fold increase in glycoprotein synthesis and production of a new glycoprotein not present in keratinizing epidermal cells. DNA synthesis is decreased as a result of this treatment yet cell number is stationary or increases, suggesting an increased life span in vitro. Radioactively labeled retinyl acetate binds to epidermal cells for at least 7 days in this in vitro system but specific intracellular localization could not be determined. In serum free short-term epidermal culture systems, retinyl acetate increased total RNA content of cells significantly. This effect was enhanced by including insulin and hydrocortisone in the culture medium. The mouse skin system thus offers parallels to respiratory and glandular epithelium in its response to agents known to effect differentiation and carcinogenesis of epithelial cells.

This Section has also continued its investigations into the interaction of chemical carcinogens with cellular macromolecules. In order to determine whether specificity is present in the reaction of carcinogenic polycyclic hydrocarbons with DNA, chromosomes have been isolated from Chinese hamster cells which were exposed to radioactively labeled carcinogens. Preliminary evidence suggests that certain chromosomal subunits composed of heterochromatic portions of the genome may bind more carcinogen than certain

euchromatic portions of the genome. Mechanistic studies in this Section are being conducted using mouse skin cell cultures. The repair of DNA damage caused by skin carcinogens β-propiolactone (BPL) and N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) was studied using tritiated deoxyribonucleosides. At low doses of each chemical, which allow for cell survival, only the DNA base attacked by these agents, guanine, is specifically inserted in the repair process. With higher doses, at which survival is minimal, all precursors are replaced. Thus, specific guanine repair may be more relevant to cell survival after damage by MNNG and BPL. This type of repair was not detectable in human WI 38 cells treated with MNNG.

The role of DNA replication at the time of initiation of carcinogenesis is also being studied using the mouse skin painting model in vivo. The application of croton oil to mouse skin results in a marked stimulation of DNA synthesis 18 hours later. If urethane is given 1 hour before or 6 or 24 hours after croton oil stimulation there is a 3-fold increase in papilloma yield. If urethane is given 6 or 12 hours before croton oil, however, there is only a 2.3- and 1.7-fold increase respectively. If urethane is given 24 hours before croton oil, there is no change in tumor incidence. With MNNG as initiator, croton oil applied 6 hours before 1, 4, 8, or 20 hours after MNNG increased the papilloma yield 2-3-fold at all times. This suggests that cell proliferation several hours after the initiator is applied may be important in tumor initiation.

Perinatal Carcinogenesis Section *"(1) Investigates the induction of cancer in experimental animals before birth and during infancy, with the goal of identifying factors responsible for the initiation and growth of tumors of childhood and infancy in man. (2) Utilizes techniques of chemical synthesis, biochemistry, histopathology, immunology and endocrinology to identify carcinogens to which, both individually and in combination, the fetus and neonate are particularly vulnerable, and to identify host factors which qualitatively or quantitatively modify their biological effects. (3) Develops measures for prevention of cancer in children or in later life as a response to conditions of high susceptibility to carcinogens during fetal life or childhood."*

The polynuclear aromatic hydrocarbon carcinogen 7,12-dimethylbenz[a]-anthracene (DMBA) has been shown to be a potent transplacental carcinogen in the Sprague-Dawley rat, with major effects on the central and peripheral nervous system, the blood vascular system, and the kidneys. Among the kidney tumors observed are true nephroblastomas, as well as a category of apparently mesenchymal tumor which often contains metaplastic squamous epithelium and smooth muscle. These tumors can also be readily induced by neonatal administration of other compounds, notably cycasin, and efforts are underway to determine whether organ cultures of these tumors can respond to embryonic inducers of renal differentiation, which would confirm their relationship to the nephroblastomas.

The rarity of cerebellar tumors in rats, in comparison with the high susceptibility of the species to tumor development in other portions of the central nervous system, contrasts markedly with the susceptibility of mice

to development of true medulloblastomas of the cerebellum as a consequence of exposure to 1-ethyl-1-nitrosurea (ENU). In collaboration with Prof. Wolfgang Wechsler of the Max-Planck-Institut für Hirnforschung in Cologne, Germany, transplantable neurogenic tumors of the mouse are being established, and ultrastructural and biochemical characterization of cultured, cloned cell lines is being undertaken. Continuous cell cultures of neurinomas have been established, and have been shown to contain protein S-100, which is specific for the nervous system. The neoplastic Schwann cells show many of the characteristics of their normal counterparts, including a tendency preferentially to coat a surface such as that of another type of co-cultivated cell. Efforts are underway to extend these studies to glial, ependymal, neuronal, and medulloblastoma cells. The mouse appears at present the most promising species for study of an animal equivalent to human medulloblastoma.

Transplacental carcinogenic studies in the Old World monkey, Erythrocebus patas, have shown that pregnant females tolerate, without interruption of pregnancy, up to 6 successive intravenous injections of ENU, each of 0.1 mg ENU/kg body weight, for a total weight-adjusted dose which is at least 10 times that necessary for a 100 per cent incidence of nerve tumors in rats. No tumors of any kind have been observed in monkeys exposed to this regimen in utero and observed up to 18 months after birth, or necropsied after this length of time following death from infectious disease. On the basis of this data it seems unlikely that ENU will induce a high incidence of the pediatric types of human neoplasms appearing early in postnatal life. Pharmacodynamic studies will be carried out to compare maternal-fetal transport and distribtuion of the carcinogen in primate versus rodent species. This work is in collaboration with Dr. W. T. London, NINDS.

Soft-tissue sarcomas were induced by water-soluble polycations in NIH General Purpose (GP) mice as previously reported. C-type RNA viruses isolated from tissue cultures of these tumors include viruses having both B and N tropisms and belonging to both Gross and FMR sub groups. While these agents are leukemogenic in GP mice, continuing efforts to identify sarcomagenic activity in these preparations are still unsuccessful. Thus, while cells of these animals are clearly capable of generating infectious C-type particles which may be involved in the regression-prone lymphomas induced by ENU in GP mice and in the high (30 per cent) incidence of lymphomas which develop in untreated animals of this line during the second year of life, there is no evidence to connect them with the sarcomagenic activity of soluble polycations. Combinations of such soluble polycations as poly-D or L-lysine with poly I:poly C have recently been shown to be extremely effective interferon inducers in primate species, since they efficiently protect the synthetic RNA polymer from degradation by serum nucleases. Collaborative studies with Drs. H. Levy and S. Baron, NIAID, are continuing on the feasibility of such polymer complexes for therapy of life-threatening viral infections in man. Since polycations are a component of this complex, further study of their carcinogenic activity is being continued.

Some transplacentally induced lung tumors in mice of genetically resistant strains are highly immunogenic in the strain of origin; grow preferentially

in F_1 hybrids between the parental strain of origin and genetically susceptible strain A; are cross-reactive with normal lung tissue of strains of mice genetically susceptible to these neoplasms; and appear to be cross-reactive with normal embryonic tissue of genetically resistant strains of mice. The antigen therefore probably has much in common with the carcino-embryonic antigens observed in both human and experimental animal tumors of certain organ systems. Approximately 40 tumors have been established from different resistant strains of mice for further studies of cross-reactive lung tumor antigen. Many of these tumors on primary isolation do not display preferential growth in susceptible F_1 hybrids. Accordingly, neuraminidase treatment is being undertaken in an effort to unmask this antigen, and to explore the extent to which it may play a role in genetically determined susceptibility to specific neoplastic disease.

The Ultrastructure Unit conducts research in chemical and physical carcinogenesis through the use of electron microscopic techniques, advises and assists staff of the Carcinogenesis Program on proper utilization of such techniques for solution of experimental problems, and instructs technical and professional staff in the quantitative aspects of autoradiography. This Unit serves as a resource to collaborate with other Sections and Units and with other Branches. Research based on the output of this Unit is reported under the Sections and Units for which the services are provided. Examples of intramural collaboration include the following projects: 1) assessing DNA repair synthesis (Digestive Systems Carcinogenesis Section, Experimental Pathology Branch; Lung Cancer Branch); 2) determining the binding of carcinogens to substructures of chromatin (In Vitro Pathogenesis Section, Experimental Pathology Branch); 3) identifying receptors of hormones and polynuclear hydrocarbons in the rat mammary gland (Endocrine Carcinogenesis Section, Experimental Pathology Branch); 4) studying the development of the rat prostate gland (Endocrine Carcinogenesis Section, Experimental Pathology Branch); 5) measuring the rate of cell proliferation in respiratory epithelium following exposure to carcinogens (Lung Cancer Branch); 6) studying localization of ^{3}H-carcinogens in respiratory epithelial cells by quantitative autoradiography; (Lung Cancer Branch); and 7) studying localization of ^{3}H-vitamin A compounds and morphological changes caused by those compounds in cultured epidermal cells (In Vitro Pathogenesis Section, Experimental Pathology Branch; Lung Cancer Branch).

Serial No. NCI-4508
1. Experimental Pathology Branch
   Carcinogenesis, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cellular Responses to Carcinogens: DNA Repair Mechanisms

Previous Serial Number: Same

Principal Investigator: Michael W. Lieberman, M.D., Ph.D.

Other Investigators: Miriam C. Poirier, M.S. and Curtis C. Harris, M.D.

Cooperating Units: Lung Cancer Branch, NCI

Man Years:

Total	:	2.1
Professional	:	1.1
Other	:	1.0

Project Description

Objectives:

As indicated in the 1973 progress report, our objectives are to assess the role of DNA repair mechanisms in carcinogenesis. Emphasis has been placed on two areas: 1) The intragenomal and intracellular distribution of DNA repair synthesis in cultured mammalian cells, with emphasis on human cells; 2) The accuracy of repair processes in restoring the damaged area.

Methods Employed:

The methods are as outlined in the 1973 progress report with several important additions. In addition to the WI-38 human diploid fibroblast line, we have utilized the Balb/c 3T3 line. The Ag^{+}-Cs_2SO_4 gradient method mentioned in last year's progress report has been published and has been used to separate mouse DNA into satellite and mainband DNA. Other techniques employed include: reassociation kinetics in solution and separation of single and double stranded DNA on hydroxyapatite; thermal elution chromatography on hydroxyapatite; S_1 nuclease digestion studies; pyrimidine isostich profile analysis; high resolution autoradiography (1 μ sections); electron microscopy.

Major Findings:

DNA repair processes are widely distributed throughout the mammalian genome. After damage with a model chemical carcinogen (N-acetoxy-2-acetylamino-fluorene) or a physical agent (ultraviolet radiation) approximately equal repair on a specific activity basis is seen in mouse satellite and mainband DNA. In the human genome, repair after damage with these two agents is present in DNAs of all degrees of kinetic complexity (i.e. repetitive and unique DNA sequences). Autoradiographic data from human cells suggests that the distribution of repair is non-uniform (i.e., that some intranuclear areas show more repair synthesis than others).

Base pairing of repair patches (the $\sim$100 nucleotides inserted after removal of thymine dimers and bulky chemical carcinogens) was examined by thermal elution chromatography and S_1 nuclease digestion. Within the limits of the techniques, it appears that bases inserted during repair synthesis are hydrogen-bonded to the template strand and represent an accurate complementary copy of it. Analysis of pyrimidine isostichs from repaired and replicated mainband and satellite mouse DNA suggest there is template specificity during repair synthesis since profiles from the same molecular species of DNA are similar to each other whether isostichs were prepared from replicated or repaired DNA.

Significance to Biomedical Research and the Program of the Institute.

It appears that a wide variety of DNA sequences which have sustained either purine or pyrimidine base damage are repairable. The best available evidence indicates that this repair results in an accurate restoration of the damaged area. The data are significant for the following reasons: 1) They suggest that failure to repair DNA of any given class of sequences (as defined by isopycnic centrifugation and reassociation rates) is probably not directly related to carcinogenesis 2) They establish that there is heterogeneity of the repair response, but leave open the nature of its selectivity; 3) They indicate that repair synthesis in mammalian cells is not grossly inaccurate (i.e., does not insert bases randomly) and is probably relatively error free. To the extent that repair synthesis is accurate, one may suggest that errors introduced during repair are not responsible for neoplastic change.

Proposed Course of Project:

With the departure of one of us (MWL) in July, 1974, a change in direction is anticipated. A more biological approach is planned. Several reports suggest that co-carcinogens inhibit DNA repair synthesis. Because the mechanism of action of co-carcinogens is largely unknown, it would be of interest to evaluate a number of co-carcinogens on a comparative basis with their non-co-carcinogenic structural analogs for their inhibitory effects on repair synthesis. The planned approach should help to evaluate previous reports and perhaps help clarify this complex biological area.

Publications

1. Lieberman, M. W.: Fractionation of mouse DNA in preparative Ag^{+}-Cs_2SO_4 gradients. Biochim. Biophys. Acta 324: 309-319, 1973.

2. Lieberman, M. W. and Poirier, M. C.: Deoxyribonucleoside incorporation during DNA repair of carcinogen-induced damage in human diploid fibroblasts. Cancer Res. 33: 2097-2103, 1973.

3. Lieberman, M. W.: Approaches to analysis of fidelity of DNA repair in human diploid cells. Inter. Rev. Cyt. suppl. 1974 (In Press).

Serial No. NCI-4510
1. Experimental Pathology Branch
   Carcinogenesis, DCCP
2. Digestive System Carcinogenesisis
   Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Induction and Development of Cancer of the Digestive System in Animals - Environmental Factors

Previous Serial Number: NCI-4618

Principal Investigator: Richard S. Yamamoto, Sc.D.

Other Investigators: Jerrold M. Ward, D.V.M., Ph.D.; Takashi Sugimura, M.D., D.M.S.; Taijiro Matsushima, Ph.D.; Takashi Kawachi, M.D., D.M.S.

Cooperating Units: Carcinogen Metabolism and Toxicology Branch
Institute of Medical Science, University of Tokyo
Japanese National Cancer Center Research Institute, Tokyo

Man Years:

Total	: 2.5
Professional	: 2
Other	: 0.5

Project Description

Objectives:

To explore the importance of specific carcinogenic compounds and co-carcinogenic environmental factors in experimental carcinogenesis of the gastrointestinal tract. The environmental factors chosen for study are suggested by the epidemiologic aspects of human gastric and colo-rectal cancer. The incidence of cancer of the stomach is high in Japan, but low and decreasing in the United States; colo-rectal cancer, however, is frequent and increasing in this country. Dietary factors are probably the main source of carcinogens in digestive system carcinogenesis, and are also considered the most likely source of cofactors. The effects of dietary factors on known stomach and colon carcinogens are investigated.

Methods Employed:

Known carcinogens, such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) for stomach cancer, and azoxymethane (AOM) and sym-dimethylhydrazine (DMH) for

colon cancer, are administered to rats and mice, in combination with dietary factors (high salt; high bulk) which may affect the response of the gastrointestinal tract to these agents. Findings are confirmed by histopathology.

Major Findings:

1. Effect of high salt diet on stomach carcinogenesis. Addition of sodium chloride (1%) to laboratory chow did not increase the incidence of adenocarcinoma of the glandular stomach when administered together with MNNG to rats. However, when fed a high salt diet after cessation of MNNG treatment, rats developed much more aggressive neoplastic lesions than those on a control diet. Crude salt, possibly containing some nitrates and nitrites, will have to be tested to determine whether this effect is due to ionic strength alone, or to contaminants in the sodium chloride.

2. Role of protease in carcinogenesis. A naturally occurring protease inhibitor, leupeptin, was tested against the production of colon cancer by AOM to evaluate a possible role of protease in the growth of tumors. Leupeptin in the diet at 0.1% decreased the incidence of tumors of the colon, but did not affect the rate of growth of those tumors which did develop.

3. The effect of leupeptin on the development of metastasis. A model system for pulmonary metastasis by Yoshida ascites hepatoma (AH 7974) was used. Leupeptin, fed or injected, reduced metastasis formation in the lungs. Injection of both leupeptin and AH 7974 cells intraperitoneally at the same time did not prevent the establishment of ascites hepatoma. There was therefore clearly an effect on either the implantation or development of metastases. Studies on the role of leupeptin in carcinogenesis and in the development of tumors are continuing in collaboration with Drs. Sugimura and Matsushima.

4. Additive effect of DMH and griseofulvin in hepatocarcinogenesis. Griseofulvin fed at a suitably high concentration or injected into newborn mice was hepatocarcinogenic by itself. DMH, at low levels which nonetheless produced colon tumors, did not produce hepatoma. Griseofulvin, a very effective antifungal antibiotic, was tested in combination with DMH to explore possible synergisms or antagonisms in the carcinogenic effects of these agents, at dose levels below that required for detectable hepatocarcinogenicity by griseofulvin alone. Animals receiving both agents did develop hepatomas, in addition to the expected incidence of colon tumors. Investigation of this co-carcinogenic effect is continuing.

Significance to Biomedical Research and the Program of the Institute.

The high morbidity and mortality from gastointestinal cancer is a major health problem in the United States. The development of animal models of

gastrointestinal neoplasms, and their use for study of etiologic factors which act in combination to modify the incidence and development of such tumors, is expected to lead to knowledge relevant to their prevention.

Proposed Course of Project:

Further studies will be made with dietary incredients, antioxidants, intestinal bacterial flora, antibiotics, resistant vs. susceptible strains of animals, enzyme inhibitors, etc., to explore their effects on carcinogenesis by compounds known to affect the gastrointestinal tract.

Honors and Awards

Japanese Government Research Award for Foreign Specialists, for a tour of duty at the National Cancer Center Research Institute in Tokyo, Japan, Oct. 1972-Sept. 1973.

Publications

Mitchell, F., Yamamoto, R. S., and Weisburger, J. H.: Griseofulvin: immunosuppressive action. Proc. Soc. Exp. Biol. and Med. 143: 165-167, 1973.

Ward, J. M., Yamamoto, R. S., and Brown, C. A.: Pathology of intestinal neoplasms and other lesions in rats exposed to azoxymethane. J. Natl. Cancer Inst. 51: 1029-1039, 1973.

Ward, J. M., Yamamoto, R. S., and Weisburger, J. H.: Brief Communication: Cellulose dietary bulk and azoxymethane-induced intestinal cancer. J. Natl. Cancer Inst. 51: 713-715, 1973.

Ulland, B. M., Weisburger, J. H., Yamamoto, R. S., and Weisburger, E. K.: Antioxidants and carcinogenesis: Butylated hydroxytoluene, but not diphenyl-p-phenylenediamine, inhibits cancer induction by N-2-fluorenylacetamide and by N-hydroxy-N-2-fluorenylacetamide in rats. Fd. Cosmet. Toxicol. 11: 199-207, 1973.

Yamamoto, R. S., Richardson, H. L., Weisburger, E. K., Weisburger, J. H., Benjamin, T., and Bahner, C. T.: Carcinogenicity of proposed cancer chemotherapeutic agents with stilbene-arylnitrosamine structures. J. Natl. Cancer Inst. 51: 1313-1315, 1973.

Ward, J. M., Yamamoto, R. S., Weisburger, J. H., and Benjamin, T.: Brief Communication: Transplantation of chemically induced metastatic mucinous adenocarcinomas of the jejunum and colon in rats. J. Natl. Cancer Inst. 51: 1997-1999, 1973.

Serial No. NCI-4507

1. Experimental Pathology Branch
   Carcinogenesis, DCCP
2. Endocrine Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Endocrine Control of Mammary Growth, Differentiation and Carcinogenesis

Previous Serial Number: Same

Principal Investigator: Douglas H. Janss, Ph.D.

Other Investigator: Mrs. Theresa Ben

Cooperating Units: None

Man Years:

Total	: 2
Professional	: 1
Other	: 1

Project Description

Objectives:

The project is designed to examine the influence of hormones on the process of mammary carcinogenesis by: examination of epidemiological data for indications of altered hormonal milieu which may be factors responsible and/or conducive either for the induction and growth or the prevention and regression of human breast cancer; elucidation of mechanisms involved in the series of hormone dependent cellular events which result in the "normal" progression of stem cell population to a differentiated cell population with specific milk synthesis function; elucidation of mechanisms whereby the exposure of the cell populations to carcinogens under the proper cellular environmental conditions alters the normal progression of cellular events, resulting in neoplastic transformation rather than in normal differentiation and function; correlation of information gained from animal and "in vitro" human tissue experiments such that model systems which adequately mimic the various aspects of human breast cancer can be developed and used to determine the cause(s) and to aid in the development of preventive measures.

Methods Employed:

Mammary epithelium (ductal and alveolar cell origin) freed from the usual large mass of surrounding adipose and dense connective tissue is utilized for in vitro and in vivo studies. Ficol gradient separation is used to yield epithelial cells free of fibroblasts. These cells are then grown in defined media. Incorporation of radioactive precursers into nucleic acids and specialized protein is used to evaluate the hormonal stimulation and altered sensitivity of both alveolar and ductal cells. These primary cell cultures are also used to examine malignant transformation resulting from exposure (in vitro) to chemical carcinogens, interaction of chemical carcinogens with cellular constituents, influence of various hormones on these interactions and the effect of these hormone-carcinogen interactions on normal cellular processes. Following various in vivo treatments, mammary epithelial cells are isolated and used to quantitate the interaction of hormones and carcinogens with cellular macromolecules. Through the use of ultracentrifugation, column chromatography and gel electrophoresis various cellular components are isolated and examined for interaction with hormones and carcinogens. Furthermore, these epithelial cells are used to study the metabolic activation of both hormones and carcinogen molecules and detoxification products formed by these cells are isolated and identified.

Major Findings:

Human breast tissue obtained following reductive mammoplasty was subjected to collagenase treatment. The resulting cellular material was centrifuged through a ficol gradient to yield ductal and alveolar epithelial cells free of contaminating fibroblasts. These cells were then grown in defined culture media. Hormone requirements have been determined. The additions of insulin, prolactin, estrogen, progesterene and hydrocostisone are required for proliferation. High resolution light microscopy and electron microscopy comparison of material prior to treatment with cell cultures indicated that the cells grown and passed in culture were histologically similar. In addition, cells with typical fibroblast histology were not observed. Using mitotic and labeling indices as criteria for cell proliferation, the quantitative hormonal requirements have been determined.

Rat mammary epithelium has been grown in culture. Both quantitative and qualitative hormone requirements have been determined. High resolution light and electron microscopy and mitotic and labeling indices have been used as means to establish hormone requirements. It was found that the fetal sera used for cell culture must be treated before use to remove certain factors (probably low levels of steroid hormones) before the effect of added hormones could be evaluated. Once this technique was perfected, the minimum steroid additions were found to elicit profound

metabolic changes. Furthermore, prior treatment of the sera reduced hormone levels below those detectable by radio-immunoassay. By using ficol gradient separation and by the addition of certain hormones both ductal and alveolar elements can be grown isolated from one another. Microscopic examination and hormonally stimulated synthesis of milk proteins indicated that these cells were epithelial and not fibroblasts. Preliminary findings indicated that the estrogen cytoplasmic protein receptor was present in these cells grown in culture.

A major effort has been expended to evaluate the influence of age on the induction of mammary tumors using 7,12-dimethylbenz[a]anthracene (DMBA) in Sprague-Dawley female rats. Following the feeding of DMBA-^{3}H to female rats of three different ages (35, 50 or 120 days of age), both mammary gland and liver were removed. DNA, RNA and soluble proteins were isolated and purified. The amount of bound DMBA-^{3}H was determined. DMBA binding to DNA isolated from rats fed at the age of highest susceptibility (50 days old) reached a maximum of 60 pmoles/mg DNA between 10-16 hours after feeding. The amount of DMBA bound to DNA decreased with time and by 42 days amounted to 18 pmoles/mg DNA. DNA isolated from rats fed when 35 or 120 days of age showed a different pattern of binding. Binding was not detectable until 10 hours, reached maximum levels by 16 hours and was not detectable after 24 days. Comparison between groups indicated that binding was depressed by an amount which was comparable to the observed depression in tumor incidence. DMBA binding to liver macromolecules was not similar to that of mammary macromolecules. The age at which DMBA was fed altered the amount of carcinogen bound to mammary DNA. This may be one of the factors affecting the incidence of mammary tumors.

Significance to Biomedical Research and the Program of the Institute:

Recently published epidemiology data indicate that the incidence of breast cancer in North American women is greater than seven times that of certain Oriental countries. Although the incidence rate in young Oriental women is similar to that of American females, the risk in American women continues to increase linearly with age while the Oriental incidence plateaus. However, Orientals who have migrated to North America or Hawaii tend to acquire the incidence characteristic of their adopted environment. Other epidemiological data suggest that the age of parity can have a significant influence on the risk of developing breast cancer. Thus, pregnancy at an early age alters the susceptibility of the breast to "carcinogenic" stimuli. Taken together it appears that many factors, i.e., physiological, endocrine, genetic, environmental, sociological, psychological and geographical may be involved in development of breast cancer. Although much is known about the induction and hormonal requirements for growth of animal breast cancer, new and/or further development and refinement of model systems are necessary to correlate human epidemiological data and animal experiments. Human material, of necessity, must be used in an

in vitro system to examine the influence of hormones and carcinogens on normal growth and differentiation and carcinogenesis. Thus, animal model systems which can be similarly studied to yield information on the mechanisms of hormonal action and carcinogen transformation must be examined. The correlation of information gained from these animal systems with the in vitro human cell experiments should add to what is presently known, so that models which adequately mimic the various aspects of human breast cancer can be developed and used to determine the cause(s) and aid in the prevention of breast cancer.

Proposed Course of Project:

A good morphological study of rat mammary epithelial growth and differentiation from infancy through old age is not available. We are continuing our studies in this area. Alveolar and ductal density in rats before, during, and after the age of maximumal susceptibility to carcinogenesis is being determined. In addition, the evaluation of mammary tissue by high resolution light and electron microscopy is nearing completion. Mitotic and labeling indices of mammary epithelium from these three ages have been completed and the data is now being evaluated. The analysis of serum levels of protactin, estrogen (estradiol, estrone and estriol) and progesterene in the 35, 50 and 120 day old rat will soon be started. These data will then be correlated with the observed changes in mammary sensitivity to these hormones. The co-factor-dependent metabolic activation and the identification of DMBA metabolites formed by epithelial cells of rats at ages both susceptible and non-susceptible to cancer induction will be undertaken. These studies should yield information which when related to our present findings will indicate the factors involved in the changing susceptibility of mammary epithelial cells to carcinogenic transformation with age.

The influence of the endocrine system on the induction of mammary cancer is well documented. Administration of high levels of hormones or drugs which stimulate the secretion of hormones which normally control mammary growth inhibits the induction of mammary tumors. However, certain levels of hormones are required for transformation. Ovariectomy prior to DMBA feeding drastically reduces tumor incidence while hypophysectomy completely eliminates the tumorigenic response. The binding of DMBA to cellular constituents following ovariectomy, hypophysectomy or the injection of hormones known to suppress tumor formation will be determined. Since the male mammary gland is not susceptible to DMBA, binding to macromolecules of the male gland will be determined. In addition, various hormone manipulations which allow for tumor formation in the male will be attempted and DMBA binding then determined. The influence of these hormonal alterations in both male and female rats on the metabolic activation and DMBA metabolite formation will be studied.

Using the techniques perfected for the culture of rat and human mammary epithelial cells, hormonal control of growth and differentiation will be studied. The interaction of hormones with cytoplasmic receptor proteins, transfer of the complexes to the nucleus and binding of the complex to DNA will be examined. The subsequent cellular functions following the binding of the hormone receptor complex to DNA will be examined. With this system it should be possible to dissect carefully the hormonal control of cellular processes which lead to cell division or cellular differentiation and specialized milk synthesis. Furthermore, in vitro transformation studies will be continued using both rat and human cells in culture. Studies attempting to elucidate hormone requirements for transformation as well as the hormones which block transformation will be undertaken. This system will also enable us to examine carcinogen-hormone interactions and their resultant effects on cytoplasmic and nuclear receptor molecules. In addition, an analysis of the cellular functions resulting from the carcinogen-hormone-receptor interaction will be studied. Using both human and rat cell cultures, correlation of growth, differentiation and carcinogenesis data can be established.

Publications

Janss, D. H., Ben, T. and Kingsbury, E. W.: Primary cell culture of normal human mammary epithelium. J. Natl. Cancer Inst. (In Press).

Serial No. NCI-4509
1. Experimental Pathology Branch
Carcinogenesis, DCCP
2. Endocrine Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Significance of Chemical-Macromolecular Interactions to Subsequent Cellular Function

Previous Serial Number: None

Principal Investigator: Beatrice P. Chen, Ph.D.

Other Investigator: H. F. Kung, Ph.D.

Cooperating Unit: Roche Institute of Molecular Biology
Nutley, New Jersey

Man Years:

Total	1.0
Professional	1.0
Other	0

Project Description

Objectives:

To explore normal and/or altered cellular functions which occur following exogenous (carcinogens) or endogenous (hormones) chemical interactions with various cellular components; to utilize these systems to elucidate the mechanisms whereby these interactions affect cellular function; to examine the modifications in cellular function induced by exogenous chemicals (carcinogens) and/or endogenous chemicals (hormones) which lead to tumorigenesis; to develop means of controlling these altered cellular processes to either prevent or reverse carcinogenesis; and to develop systems which might rapidly and accurately predict carcinogenic potentials of chemicals.

Methods Employed:

A well defined system where transcription and translation can be controlled is used to examine the effect of chemical interaction with various cellular components and subsequent cellular expression as measure by the synthesis of specific enzyme activity. The *in vitro* synthesis of a specific enzyme, β-galactosidase, from a bacteriophage DNA (λ h80p^s lac) that contains lac operon is used. The *in vitro* synthesis system has been established and has previously been employed by the principal investigator. Recently, Dr. H. F.

Kung, at Roche Institute of Molecular Biology, has purified the system to such a degree that one need only use the necessary purified factors such as RNA polymerase, high salt washed ribosomes, aminoacyl tRNA's, protein synthesis cofactors and precursor. Direct acting carcinogens are reacted in vitro with bacterial or target organ macromolecules. Those carcinogens which required metabolic activation are incubated with a microsome system plus macromolecules. In addition, macromolecules other than DNA are isolated from target tissue following in vivo carcinogen administration. Macromolecules are isolated and purified, the amount of carcinogen bound is quantitated, and the isolated macromolecules substituted for their bacterial counterparts in the in vitro synthetic system.

Major Findings:

When ^{3}H-dimethyl sulfate was incubated with λh80p^{S}DNA, a definite peak of radioactivity was associated with DNA banded in CsCl gradients. This was presumed to be the methylated DNA. The amount of methylation was directly proportional to the amount of substrate dimethyl sulfate. This DNA is now being examined for structural and functional integrity in the β-galactosidase synthesis system.

Significance to Biomedical Research and the Program of the Institute:

In the search for the mechanism of action of carcinogens, there is little information concerning the exact functional defect caused by those agents. Since one can easily replace any post-DNA component along the DNA-RNA-Protein synthesis pathway in this bacterial system, we may exchange various mammalian components for their bacterial counterparts, and possibly determine whether function is specifically impaired as a consequence of chemical binding to any one of those components.

Proposed Course of Project:

First, various conditions which may affect binding of carcinogens and hormones to DNA and other macromolecules will be examined, i.e., ionic strength, temperature, pH etc. Second, bound DNA complexes will be used to direct β-galactosidase synthesis in vitro in an attempt to ascertain possible changes in the activity of the enzyme. Various carcinogens potent or weak, will be examined for correlation with their in vitro effects in this system. Although the lac operon is required for this system, it is possible to interchange other macromolecular components. Thus, some RNAs (tRNA, ribosomes) from endocrine target organs such as prostate and mammary gland can be examined for structural-function integrity following binding with carcinogens as well as hormones.

While this bacterial system is being used, an attempt will be made to develop a mammalian system. It is well known that following specific hormonal fluctuations the mammary gland synthesis of casein is stimulated.

Using the background gained in developing the bacterial system, it should be possible to isolate and purify the mammalian systems. Epithelial cultures of mammary acinar cells will be used in our first trials. The requirements for induced casein synthesis in culture have been developed. Furthermore, techniques for the isolation, purification and quantitation of casein are available. These techniques may also be used to assay for the synthesis of an altered casein following carcinogen-macromolecule binding.

Serial No. NCI-4502
1. Experimental Pathology Branch
   Carcinogenesis, DCCP
2. In Vitro Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: The Role of DNA Replication and Repair in Chemical Carcinogenesis

Previous Serial Number: Same

Principal Investigator: Henry Hennings, Ph.D.

Other Investigators: Delores Michael and Elroy Patterson

Cooperating Units: None

Man Years:

Total	: 2.4
Professional	: 1.0
Other	: 1.4

Project Description

Objectives:

To examine the repair of DNA in mouse skin cells after carcinogen treatment; to examine the importance of cell proliferation in skin carcinogenesis initiated by urethane or N-methyl-N'-nitro-N-nitrosoguanidine (MNNG).

Methods Employed:

Cell cultures of newborn mouse skin grown in petri dishes were used for extraction of DNA by a standard phenol procedure. Bromodeoxyuridine (BUdR)-labeled DNA was separated from DNA of normal density in cesium chloride gradients in a Beckman L2-65B ultracentrifuge. In recent experiments, the cells were lysed in 1% sarkosyl, homogenized, then centrifuged in a cesium chloride-cesium sulfate gradient to separate DNA from RNA and protein.

In tumor induction experiments in susceptible female mice, skin tumors were initiated by the i.p. injection of 25 mg of urethane or the application of 4 μmoles MNNG in acetone to the dorsal epidermis and promoted by weekly applications of 0.5% croton oil in acetone. In biochemical experiments in mouse skin, the animal was sacrificed, the back skin was removed and put

into cold 1% acetic acid overnight. The epidermis could then be scraped off and used for preparation of a nucleic acid hydrolysate by cold perchloric acid precipitation of macromolecules and hot perchloric acid hydrolysis.

Major Findings:

DNA repair in primary cultures of mouse skin cells was studied by labeling newly-synthesized DNA with both 5-bromodeoxyuridine (BUdR) and a tritiated DNA precursor, separating the BUdR-containing DNA from DNA of normal density by cesium chloride density gradient centrifugation, and determining the location of tritium in DNA with respect to the two peaks of absorbance. Tritiated DNA synthesized by cells in S phase should be found only in the heavy, BUdR-containing peak, while tritium incorporated into light, unreplicated DNA indicates repair synthesis.

Exposure of cells to ultraviolet light produces pyrimidine dimers, which are excised along with 30-100 adjacent bases in the same DNA strand. Faithful repair of this DNA strand should be demonstrable with any DNA precursor. In mouse skin cells irradiated with ultraviolet light (87-174 ergs/mm^2), and incubated for 3 hours with BUdR and either thymidine-^{3}H or deoxyguanosine-^{3}H, tritium was found in the non-replicating peak of DNA, as well as in the replicating peak. Thus, these cells can carry out excision repair, as demonstrated with either a pyrimidine or a purine precursor.

Similar repair with all precursors tested was shown after treatment with high, toxic doses of the skin carcinogens β-propiolactone (BPL)(10^{-3}M) or MNNG (1.7-3.4 x 10^{-4}M). However, after treatment with lower, less toxic doses of BPL (5 x 10^{-4}M) or MNNG (3.4 x 10^{-5}M), thymidine-^{3}H and deoxycytidine-^{3}H were incorporated only into replicating DNA, while deoxyguanosine-^{3}H was incorporated into both replicating and non-replicating DNA. Thus, repair was demonstrated easily with deoxyguanosine-^{3}H as precursor. With high levels of deoxyadenosine-^{3}H, it was also possible to show repair synthesis. However, we found that in mouse skin cells, only about 50% of the tritium from AdR-^{3}H is incorporated into adenine in DNA; 47% was found in guanine. Thus, this repair synthesis may result from the incorporation of the precursor into guanine, not adenine.

Both BPL and MNNG react primarily with guanine in DNA, resulting in the loss of the alkylated guanine from the DNA strand. This loss appears to be repaired specifically by incorporation of deoxyguanosine. This apparently specific repair seen at lower, less toxic doses of carcinogen may be more relevant to cell survival than the non-specific repair seen at very toxic doses.

We have looked for guanine-specific repair after MNNG treatment of WI-38 human diploid fibroblasts, but have been unable to detect any differences

between pyrimidine and purine precursors. At 3.4×10^{-5}M MNNG, repair was demonstrated with either thymidine-^{3}H or deoxyguanosine-^{3}H. At 6.8×10^{-6}M MNNG, repair was not seen with any precursor.

Cell proliferation appears to be important in the initiation of skin tumor formation, but its exact role remains to be elucidated. Treatment with the hyperplastic agent croton oil 18-24 hours before initiation with urethane or MNNG increased the papilloma incidence in initiation-promotion experiments about 3-fold. This result had been interpreted by several authors to indicate the importance in initiation of DNA replication at the time of carcinogen application, since the peak of DNA synthesis after croton oil treatment occurs at around 18 hours. In order to determine more precisely the time at which replication may be important in initiation, we tested shorter croton oil pretreatment times, as well as times during the first day after initiator application.

With urethane as initiator (25 mg./mouse, i.p.), when croton oil was given 24 or 6 hours before or 1 hour after urethane injection, the papilloma yield was increased 3-fold. When croton oil was applied 6 or 12 hours after urethane injection, the enhancement was reduced to 2.3- and 1.7-fold, respectively. Croton oil applied at 24 hours after urethane injection did not affect the tumor yield. With MNNG as initiator, croton oil applied 6 hours before or 1, 4, 8 or 20 hours after MNNG application (4 µmoles/0.2 ml acetone) increased the papilloma yield 2- to 3-fold at all times tested.

These results suggest that cell proliferation (or DNA replication) several hours after the initiator is applied and has interacted with target molecules in the cell may be important in tumor initiation. We are presently determining the times at which DNA synthesis is stimulated when the various combinations of croton oil and MNNG or urethane are applied to the mice. Preliminary results indicate that initiator treatment delays the usual increase in DNA synthesis after croton oil application, but the extent and duration of the increase is as much or greater than with croton oil alone. The croton oil-induced increase in the rate of DNA synthesis could cause replication of alkylated DNA before it has been repaired and result in permanent DNA alterations. Alternatively, the reported inhibition of DNA repair by the phorbol esters found in croton oil could explain the enhancement of tumorigenesis by croton oil applied on the day of initiation.

Significance to Biomedical Research and the Program of the Institute:

If a carcinogen-induced alteration in DNA is necessary for carcinogenesis, then the repair of the critical alteration should reduce subsequent tumor formation. Replication of the DNA prior to repair may result in a permanent non-repairable change in the base sequence of the newly-synthesized DNA. Conversely, errors in the repair process could also lead to DNA alterations. Our experiments are designed to help define the roles of DNA replication and DNA repair in chemical carcinogenesis.

Proposed Course of Project

DNA repair will be examined in cultures of epidermal cells and of fibroblasts to determine whether one or both types of cells are responsible for the DNA repair already seen in mixed epidermal-fibroblast cultures. Croton oil (or an active phorbol ester) will be tested to see whether it inhibits excision repair or guanine-specific repair in mouse skin cells. The extent of DNA repair will be compared in rapidly and slowly proliferating skin cells.

Pregnant mice have been injected with urethane in the last day of pregnancy to determine whether skin tumor initiation has been accomplished in the offspring. DNA repair will be examined in skin cultures prepared from the offspring which were born about 12 hours after urethane injection of the pregnant mouse. DNA repair will also be examined in cell cultures treated with chemically reactive derivatives of urethane. Tumor induction experiments will be designed to determine whether croton oil or other hyperplastic agents such as acetic acid enhance tumor initiation by initiators other than urethane and MNNG, such as dimethylbenzanthracene or β-propiolactone. The mechanisms of action of several inhibitors of skin tumor formation (poly I.poly C, sodium selenide, griseofulvin, vitamin A, protease inhibitors, dexamethasone and hydrocortisone) will be examined.

Publications

Hennings, H.: The epidermal chalone and keratinizing epithelia. In Forscher, B. K. and Houck, J. C. (Eds.): Chalones: Concepts and Current Research. Natl. Cancer Inst. Monogr. No. 38, Wash., D. C. U.S. Govt. Print. Off., 1973, pp. 69-70.

Hennings, H., Michael, D., and Patterson, P.: Enhancement of skin tumorigenesis by a single application of croton oil before or soon after initiation by urethan. Cancer Res. 33: 3130-3134, 1973.

Houck, J. C. and Hennings, H.: Chalones: Specific endogenous mitotic inhibitors. Fed. Eur. Biochem. Soc. Lett. 32: 1-8, 1973.

Hennings, H., Michael, D., Eaton, S. and Morgan, D. L.: Purine-specific repair of β-Propiolactone-induced DNA damage in mouse skin cells. J. Invest. Derm. (In Press)

Serial No. NCI-4504

1. Experimental Pathology Branch
   Carcinogenesis, DCCP
2. In Vitro Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Model Systems for the Study of Chemical Carcinogenesis at the Cellular Level

Previous Serial Number: None

Principal Investigator: Stuart H. Yuspa, M.D.

Other Investigators: Paul E. Dermer, M.D. and
David L. Morgan, B.S.

Cooperating Units: Lung Cancer Branch

Man Years:

Total	:	2.5
Professional	:	2.5
Other	:	0

Project Description

Objectives:

To develop model systems which can be utilized to study cellular events which occur during early stages of chemical carcinogenesis. Systems studied are directed to give both general information regarding malignant transformation within a mammalian cell and specific requirements of specialized cells which might shed light on events necessary for tumor development in a particular organ.

Methods Employed:

Systems have been developed for studying epithelial cell transformation in vitro. A model system of mouse epidermal cell culture which parallels the in vivo model of mouse skin carcinogenesis has received most attention. Newborn mouse skin is floated overnight on a cold trypsin solution which separates dermis from epidermis. Epidermal cells can then be dissociated and grown as a monolayer. Hair follicles and dermal fibroblasts can also be isolated and grown in culture. Epidermal cells can be grafted back to syngeneic hosts as a skin graft on graft beds prepared several weeks

earlier and protected by silicone domes and wire screens. Epidermal cells can be treated by chemical and viral carcinogens in vitro and continued in vitro for long-term studies or grafted back to hosts for in vivo passage. In addition promoting agents can be applied to epidermal cells in vitro. Treated cultured cells are evaluated by several criteria: morphology by both the light and electron microscope, growth in agar, and injection back into mice. Since newborn epidermal cells are utilized, pregnant mice can be injected with carcinogens and the epidermis of the offspring cultivated in vitro. This gives an approach to the study of promotion in vitro. Epidermal cell kinetics are also being studied using radioactive labeling with DNA precursors and isolation of DNA.

Epidermal cell differentiation is being studied in this system in order to relate this phenomenon to carcinogenesis. Vitamin A treatment of these cells caused a marked alteration in differentiation. This is observed by light and electron microscopy, DNA turnover studies, glycoprotein synthesis evaluation, autoradiography and biochemical interaction studies between chemical carcinogens and altered cells. This system is being used to alter the response in vitro between carcinogens and epidermal cells. The interaction of chemicals with cellular macromolecules is being studied in isolated chromosomes. Chromosomes are isolated from Chinese hamster cells and processed into sub-chromosomal units by salt extraction. Binding of carcinogens to these subunits are studied using ultracentrifugation, spectrophotometric, and radiotracer techniques.

Major Findings:

Mouse epidermal cells, free of fibroblasts, can be grown in vitro and will keratinize as they do in vivo. These cells can be grafted back to syngeneic F_1 hybrid hosts and will form skin characteristic of the donor strain. Cell kinetic studies of epidermal cells in vitro suggest a pattern of partially synchronized DNA synthesis occurring on the second and the eighth and ninth in vitro days. Total cell number decreases steadily as cells keratinize and slough. Ultrastructurally these cells contain tonofilaments, desmosomes and granules suggestive of keratohyalin. Cells from early primary cultures will bind polycyclic hydrocarbon carcinogens to cellular macromolecules; but by 9 days in vitro, at a time of high DNA synthesis, this ability is markedly decreased or absent. The promoting agent 12-O-tetradecanoyl-phorbol-13-acetate (TPA) will stimulate cell division in vitro. This phorbol ester effect on the epidermal cells is altered by pretreating cells with 7,12-dimethylbenz[a]anthracene (DMBA).

If pregnant mice are treated intraperitoneally with urethane and their offspring used as a source of epidermal cells, the pattern of DNA synthesis is altered. There is a marked stimulation of replication and the lifespan of epidermal cell cultures is increased. Combined treatments of urethane in vivo and TPA or DMBA in vitro has yielded cell lines apparently transformed morphologically and biologically. Tumorigenicity studies are in progress.

Retinyl acetate (RA) added to the tissue culture medium in DMSO causes marked alteration in epidermal cell differentiation. Keratinization is inhibited. There is a two-fold increase in glycoprotein synthesis with production of a new species of glycoprotein as compared to controls. Although DNA synthesis is not increased, total cell number remains unchanged or increases in the presence of RA suggesting a prolonged lifespan. Repair of DNA damage caused by ultraviolet light is unaltered in RA treated cells. Retinyl acetate restores the ability of epidermal cells to bind DMBA to macromolecules after 10 days of culture. RA remains bound to epidermal cells for up to 7 days after a single 18 hour exposure.

Chinese hamster chromosomes can be isolated in large numbers from cells in mitosis, harvested after a colcemid block. Polycyclic hydrocarbon carcinogens will bind to the macromolecules of these chromosomes. Chromosomes can be fractionated by size, or alternatively, chromosomes can be fractionated into subunits by salt extraction. This technique yields a structural chromosomal unit and a functional chromosomal unit, the former being heterochromatic and the latter euchromatic. Studies to investigate whether preferential binding of carcinogens occurs in a chromosomal subunit are in progress.

Significance to Biomedical Research and the Program of the Institute:

It is essential to understand early changes at the cellular level which occur during initiation of malignant change in order to prevent this transformation from occurring or to revert cells from an initiated state back to normal. The use of the epidermal cell culture system developed in this laboratory has certain advantages in approaching this problem. It utilizes epithelial tissues which we have shown are normal in terms of differentiation and ability to perform properly when placed back into its environment in the whole animal. The development of an in vitro skin carcinogenesis model allows for the continued study at a cellular level of extensive in vivo data which could not be carried further due to limitations of the whole animal. The separation of skin components allows for the first time a model to determine which cell types (i.e. hair follicle, basal cell, fibroblasts) are essential for the transformation to occur. With transplantation at an observable animal site performed at an early state in the in vitro life of the cells, the long-term ambiguities of cell culture can be avoided. The use of retinyl acetate in this culture system allows a systematic approach to the study of differentiation and vitamin A effects.

There is a growing but ambiguous literature on both vitamin A effects on carcinogenesis and the relationship between differentiation and carcinogenesis. This should be amenable to study using this epidermal cell culture system. Evidence from other systems suggest that vitamin A may be able to reverse the process of malignant transformation in initiated cells. If this process could be understood or studied more extensively it might offer an opportunity to alter the prognosis for some categories of human individuals at high risk for carcinoma.

The combined use of transplacental treatment of epidermal cells with in vitro observation and manipulation allows several potentially important new uses for this system. The process of promotion can be studied in vitro in cells initiated prior to culture. Preliminary evidence suggests that the addition of a second carcinogen in vitro causes a rapid transformation to occur. This is potentially useful then as an assay system for both promoting agents and initiating agents.

There is increasing evidence that chemical carcinogens alter genetic material. Chromosomes and sub-chromosomal units offer a way of studying intact genetic information. The effects of chemical agents on those structures should be of great interest.

Proposed Course of Project:

Epidermal cells will be used to study carcinogenesis in vitro or by combined in vitro and in vivo techniques. The role of state of differentiation, proliferative capacity, and cell cycle will be studied. Both normal and preinitiated cells will be used. Phorbol ester will be added to initiated cells to study promotion in vitro.

The role of vitamin A in carcinogenesis will be studied in this system. An attempt will be made to separate effects on initiation from effects on promotion. Parallel in vivo studies will be performed.

Binding of carcinogens to informational subunits of the cell will be studied further. When studies on chromosomal subunits are completed, groups of chromosomes will be isolated by size and binding will be studied.

Publications

1. Yuspa, S. H., Morgan, D. L., and Levy, J. A.: In vitro cultivation of a chemically induced epidermal carcinoma: Establishment of three cell lines and isolation of murine leukemia virus. J. Natl. Cancer Inst. 50: 1561-1570, 1973.

2. Sporn, M. B., Dunlap, N. M., and Yuspa, S. H.: Retinyl Acetate: Effect on cellular content of RNA in epidermis in cell culture in chemically defined medium. Science 182: 722-723, 1973.

3. Elias, P. M., Yuspa, S. H., Gullino, M., Morgan, D. L., Bates, R. R., and Lutzner, M. A.: In vitro neoplastic transformation of mouse skin cells: Morphology and ultrastructure of cells and tumors. J. Inv. Derm. (In Press).

4. Yuspa, S. H. and Harris, C. C.: Altered differentiation of mouse epidermal cells treated with retinyl acetate in vitro. Exp. Cell Res. (In Press).

5. DeLuca, L. and Yuspa, S. H.: Altered glycoprotein synthesis in mouse epidermal cells treated with retinyl acetate in vitro. Exp. Cell Res. (In Press).

Serial No. NCI-4506
1. Experimental Pathology Branch, Carcinogenesis, DCCP
2. Endocrine Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Further Developmental Models of Prostatic Carcinogenesis

Previous Serial Number: Same

Principal Investigator: Winston D. Edwards, Ph.D.

Other Investigators: Lawrence Lanier; Martin Wenk, Ph.D., Elizabeth Kingsbury, Ph.D.

Cooperating Units: Bionetics Research Laboratories
Microbiological Associates

Man Years:

Total:	2.5
Professional:	2.0
Other:	0.5

Project Description

Objectives:

These experiments are aimed at providing information about the morphology, mitotic activity, metabolic activity (DNA synthesis), and endocrinology of the prostate as they affect carcinogenesis in that organ. These are designed to aid in the development of an experimental model of prostatic carcinoma.

The principal experimental effort in 1974/75 will be directed to organ culture of the prostate. To be examined are the direct effects of carcinogens on the cell populations which are of immediate relevance for development of an animal model of human prostatic adenocarcinoma.

Methods Employed:

Techniques for the transplantation of prostate pieces to the cleared 4th mammary fat pad of the Balb/c mouse (see "major findings") have been developed. These prostate pieces (nodules) are now being removed from the fat pad sites and grown *in vitro*. The explants will be treated with

hormone and/or carcinogens in organ culture. Three carcinogens (7,12-dimethylbenz[a]anthracene (DMBA), 3-methylcholanthrene (MCA), and 1-ethyl-1-nitrosourea already used in the in vivo systems will now be tried in the in vitro studies.

The methods employed in our preliminary studies to provide information about the morphology and differentiation between the prostatic lobes will now be used in the organ culture systems. These methods are: mitotic index (colchicine), autoradiography, DNA turnover studies using tritiated percursors of DNA and standard DNA extraction, histology, high resolution microscopy, and electron microscopy.

Major Findings:

Morphological and histological differentiation between the prostatic lobes has been established. Knowledge of the basic (fine) structure of the prostate has been gained by examination at high resolution light microscopy and electron microscopy in prenatal, neonatal, pre-pubertal, and adult stages. The mitotic activity of epithelial cell populations in prostatic lobes of rats (F344) and mice (Balb/c) of various ages has been studied and autoradiographs of prostatic tissue labeled with tritiated thymidine to demonstrate DNA synthesis in epithelial cell populations of the prostatic lobes of F344 rats and Balb/c mice of the different age groups have been made. A study of the weight increase of the prostate for the various age groups has also been completed.

A suitable site for growing prostate transplants has been found. Pieces of prostate transplanted to the cleared 4th mammary fat pad of 2-3 week old Balb/c mice have survived for more than 18 months. Histological examination of these transplants showed that the epithelial height was maintained, there was increased secretory activity following treatment with testosterone, and the stoma increased in both cell and fiber content.

All three prostatic lobes of the rat and both the ventral and dorsal lobes of the mouse have been successfully grown (3-7 days) in organ culture. After seven days the organs still exhibited normal epithelial height and secretory activity. Organs cultured in the absence of testosterone were not maintained. Recent reports have revealed the presence of a considerable amount of spermine and spermidine in the rat's prostatic fluid. Consequently, an attempt has been made to improve the cultivation of prostate by including spermine and spermidine in the media.

Based on the morphological findings in our early work the following investigations have been in progress for more than one year:

A. The effects of hormones (estrogen, prolactin, testosterone) etc. on morphology, mitosis, and carcinogenesis of the prostate.

B. The effect of excess mating on prostatic cancer.

C. Direct injection of carcinogen (1-ethyl-1-nitrosourea) into prostate to induce adenocarcinoma. A case of prostatic adenocarcinoma was recently observed in such an animal.

These studies on excess mating, direct injection of carcinogens into the prostate, and investigation of the effects of hormone stimulation on prostatic carcinogenesis are in progress.

Other carcinogens (i.e., DMBA and MCA) have been injected directly into prostates and into prostate transplants in the cleared 4th mammary fat pads. Several tumors have been induced, but because the histological examination of these tissues is still incomplete detailed findings cannot be reported at present.

In addition to the experiments attempting to induce prostatic adenocarcinoma already outlined in this report the following studies have also been started:

a. Studies on carcinogenesis of the prostate in old male rats (retired breeders and non-breeders).

b. Influences of hormones on prostate differentiation and function.

c. Changes in prostate sensitivity to hormones with aging.

Significance to Biomedical Research and the Program of the Institute:

Despite the fact that the prostate is one of the most common sites of cancer in the male, there are at present no experimental models for this kind of cancer. The development of a suitable animal model for studies of prostatic adenocarcinoma and attempts to demonstrate similarities to and differences from the human gland are immediate goals of this project.

Proposed Course of Project:

For the year 1974/75 this project shall be directed to the following:

A. Publication of all experiments completed in 1972-73.

B. Continuation of all experiments undertaken with the cooperation of Bionetics Research Laboratories and Microbiological Associates (i.e. experiments attempting to induce prostatic adenocarcinoma outlined in this report).

C. Prostatic Cancer has been called the "Cancer of Old Men" and the age "fifty" as the age after which prostatic cancer is most frequent. Although spontaneous prostate adenocarcinoma has been rarely observed in the rat, a study of older animals would seem appropriate in

experiments aimed at comparing the animal/man situations. To this end, the studies of the carcinogenesis of prostates in older rats and mice started in 1972 are being continued.

D. Employing organ culture methods already being used in our laboratory, the following studies will be undertaken:

1. Differentiation and its relationship to carcinogenesis.

2. Relationship between hormone dependence and carcinogenesis.

3. Effects of vitamin A (anti-carcinogen) on transformation.

Serial No. NCI-4501
1. Experimental Pathology Branch
Carcinogenesis, DCCP
2. Perinatal Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Transplacental and Neonatal Carcinogenesis in Rats: An Experimental Model of Wilms' Tumor

Previous Serial Number: Same

Principal Investigator: Sewa Ram Joshi, B.V.Sc., Ph.D.

Other Investigators: Jerry M. Rice, Ph.D.; Martin Wenk, Ph.D.; Ray Shenefelt, M.D.; Larry Keefer, Ph.D.; Peter Roller, Ph.D.

Cooperating Units: Microbiological Associates, Inc., Bethesda, MD
University of Arkansas Medical Center, Little Rock, AR
Toxicology and Metabolism Branch, DCCP, NCI

Man Years:

Total	:	2.0
Professional	:	1.5
Other	:	0.5

Project Description

Objectives:

To investigate the transplacental carcinogenic activity of chemicals in the rat, and to develop in that species an experimental model for Wilms' tumor of the kidney. The model, once established, will be used for studies of physiological factors which modify induction of this neoplasm. The goal of the transplacental studies is to define the potential of environmental carcinogens and their metabolites for induction of human tumors of infancy and childhood.

Methods Employed:

Organic compounds, both carcinogenic and non-carcinogenic, are synthesized or purified from commercial sources using standard procedures. Rats of various strains are exposed to these compounds at various times during pre- and postnatal life, and the carcinogenic response in various organs, especially the kidney, is evaluated histologically. Surgical techniques are used for transplantation of tissues and removal of endocrine organs to

modify hormonal balance. Organ culture techniques are being undertaken to determine the ability of apparently mesenchymal rat kidney tumors to respond to embryological inducers of renal differentiation.

Major Findings:

Transplacental 7,12-dimethylbenz[a]anthracene (DMBA) in the Sprague-Dawley rat has induced tumors of the kidney, central and peripheral nervous systems, and blood vascular system in more than 50 percent of the animals at risk in less than one year. Rats exposed in utero were foster nursed immediately on untreated females to restrict carcinogen exposure to the prenatal period. Neurogenic tumors included neurinomas of cranial and spinal nerves; astrocytomas, ependymomas, oligodendrogliomas, and primary neurogenic sarcomas of the cerebrum and mid-brain; and severe congenital disorganization of the granular layer of the cerebellum, but only one cerebellar tumor, a poorly differentiated oligodendroglioma. Renal tumors included clear-cell and papillary cystadenomas, nephroblastomas, sarcomas, and so-called "interstitial cell" tumors.

These "interstitial cell" tumors, while lacking epithelial elements, sometimes contain metaplastic squamous epithelium and smooth muscle, in the fashion of human nephroblastomas. Experiments are in progress to determine whether organ cultures of such tumors can respond to embryonic inducers (such as the fetal mouse spinal cord) of renal differentiation, and thus establish their relationship to overtly nephroblastic tumors.

The extremely weak transplacental carcinogenic activity of the dialkylnitrosamines, such as dimethylnitrosamine (DMN) in marked contrast to the powerfully active alkylnitrosoureas, has been ascribed to the failure of fetal tissues to provide the enzyme-mediated metabolic conversion of DMN and related compounds to chemically reactive carcinogenic metabolites. Synthesis of stable derivatives of α-hydroxy dialkylnitrosamines has been achieved by members of the Toxicology and Metabolism Branch, and in collaboration with them the comparative toxicity and carcinogenicity of DMN, methyl acetoxymethylnitrosamine, and methyl methoxymethylnitrosamine are being established in fetal, newborn, and adult Sprague-Dawley rats.

Significance to Biomedical Research and the Program of the Institute:

Research for animal models of human childhood neoplasms should provide an insight into the types of causative agents and modes of exposures responsible for childhood cancer. It is to be expected that natural selection would tend to eliminate genotypes in the human population which predispose individuals to the development of fatal neoplasms before attaining reproductive age, yet the incidence of tumors in childhood is high. Epidemiological studies have pointed to the occurrence of childhood neoplasms in association with certain types of congenital malformation which are non-inherited, and suggest that

environmental agents, alone or in combination, may play a role in the induction of such neoplasms. Animal models would allow laboratory study of factors which epidemiological studies on man suggest are important in the genesis of these tumors.

Proposed Course of Project:

Rat renal "interstitial cell" tumors will be studied in organ culture to determine whether or not the morphologically mesenchymal tumors can be induced to form characteristic renal tubules and glomeruli, thus confirming the identification of such neoplasms as nephroblastomas. Once the model is established, it should be used to determine the effects of manipulations of physiological parameters, including hormonal balance and immunological responsiveness, on the genesis and behavior of these tumors. Studies should also be made to determine what synergisms may exist between carcinogens and other non-oncogenic teratogens.

Major attention should resume on the production of viable offspring in various inbred strains of rats which have urogenital malformations as a consequence of maternal vitamin A deficiency during pregnancy. This is accomplished by raising female rats from birth on a vitamin A deficient diet in order to deplete body reserves of the vitamin and then maintaining the animals during pregnancy on just enough retinoic acid or retinoic acid supplemented with retinyl acetate to maintain pregnancy. Rats born to vitamin A deficient mothers will be exposed both in utero and neonatally to cycasin and to nitroso compounds in order to determine whether we can duplicate in the experimental rat system, the observed association in man of urogenital malformations, aniridia, and other congenital defects (known to be inducible in the rat by vitamin A deficiency) with an excess occurrence of Wilms' tumor.

The transplacental hydrocarbon carcinogenesis studies should be extended to environmental hydrocarbon carcinogens, notably benzo[a]pyrene.

Serial No. NCI-4503
1. Experimental Pathology Branch
   Carcinogenesis, DCCP
2. Perinatal Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Immunological Factors Responsible for the Differential Sensitivity Between Fetal and Adult Mice, and Between Different Inbred Strains, to the Induction of Pulmonary Tumors

Previous Serial Number: Same

Principal Investigator: Jerry M. Rice, Ph.D.

Other Investigators: W. Graeme Cotton, D.V.S.M., Ph.D. and W. John Martin, M.D., Ph.D.

Cooperating Units: Immunology Branch, NCI
St. Bartholomew's Hospital, London, England

Man Years:

Total	3.5
Professional	2.5
Other	1.0

Project Description

Objectives:

To determine the manner and the extent to which immunological factors are responsible for the genetically determined differences in sensitivity to the induction and growth of pulmonary tumors among different inbred strains of mice, and between the fetus and adult within each strain.

Methods Employed:

Tumors are induced transplacentally by treatment of timed-pregnant mice with 1-ethyl-1-nitrosourea (ENU), which is synthesized in this laboratory. Tumors are transplanted to secondary hosts using trocar implants or counted cell suspensions, and to serial cell culture using standard media and procedures. Microcytotoxicity tests are carried out using lymph node and spleen cells from tumor bearing animals. *In vivo* studies of immunological cross-reaction are carried out by pre-immunizing mice with normal or tumor tissue, subsequently suppressing the primary (but not the secondary) immune response by 400 R whole-body X-irradiation, and challenging with the tumor to be tested.

Masked cell surface antigens are exposed by digestion of viable cells with neuraminidase or other enzymes.

Major Findings:

Some, but not all, pulmonary tumors induced transplacentally in strains of mice genetically resistant to these tumors grow poorly or not at all on transplantation to syngeneic recipients, but readily in F_1 hybrids between the strain of origin and another strain genetically more susceptible to the induction of the same type of tumor. Two such tumors from strain C3Hf, which is moderately resistant to pulmonary tumor induction, behave in this fashion and have been shown to be cross-reactive with each other and with normal lung tissue from genetically sensitive strain A, but not with a histologically unrelated tumor (sarcoma) or with lung tissue from genetically resistant strain DBA/2. Neither tumor is transplantable to another inbred strain in the absence of prior immune suppression, with the single exception that both grow extremely well in strain C3H, from which strain C3Hf was derived prior to 1950. Strains C3H and C3Hf reject reciprocal skin grafts and have been shown to differ from each other at non-H-2 histocompatibility loci. Normal lung tissue from strain C3H can immunize C3Hf animals against C3Hf lung tumor 85. This tumor grows uniformly well in strain C3H, the F_1 hybrid between the two strains, and in 80 percent of the F_2 generation, indicating that a single gene is responsible for the unexpected preferential growth of this tumor in an allogenic host. We conclude from studies such as these that genetically controlled sensitivity to pulmonary tumors in mice depends in part on whether the host animal can distinguish antigens characteristic of pulmonary tumor cells from its own normal lung tissue.

Extension of these studies to approximately 40 new transplacentally induced lung tumors in strains (C3Hf, DBA/2, and C57Bl/6 (all resistant to lung tumor induction and growth) and to 5 spontaneous lung tumors in susceptible strain NGP have shown only 2 in which the same hybrid preference has appeared in the first transplant generation using simple trocar implants. On the other hand, in all the resistant strains a number of lung tumors have clearly shown syngeneic preference, i.e., a classical hybrid effect with preferential growth in the parental strain of origin. Studies are being undertaken to determine whether the common antigen in strain A lung tissue and in lung tumor 85 can be unmasked in such tumors by treatment with neuraminidase and other enzymes.

Preliminary studies indicate that immunization of C3Hf mice against tumor 85 can be effected by normal fetal, but not adult tissues of lung tumor resistant strain C57Bl/6. This suggests that the antigen behaves like many othe types of carcino-embryonic antigens, in that it is normally expressed on cell surfaces at some time during development, but appears in adult tissues only under certain abnormal conditions, especially neoplasia.

Transplacentally induced hepatocellular tumors, which occur readily in strain C3Hf and less frequently in other strains, have been transplanted from strains DBA/2, C57Bl/6, and A to recipients of the parental strain and its F_1

hybrid with strain C3Hf, to ascertain whether an inverse hybrid effect can be observed with liver tumors. These are preliminary experiments, so far yielding insufficient data for comment.

Significance to Biomedical Research and the Program of the Institute:

This experimental system represents the first experimental demonstration of a role of immune factors in genetically determined sensitivity and resistance to a particular type of tumor. The existence of cross-reactive antigens on tumors and normal lung tissue of susceptible individuals invites the question of whether a similar mechanism may explain tolerance (susceptibility) to other types of tumors in animals or in human beings. This in turn is of significance to any efforts to develop immunotherapeutic measures against neoplastic disease, since the problem of avoiding autoimmunization must be faced. This experimental model provides the means for exploring the significance of that problem, as well as of determining whether the non-specific stimulation of immune responsiveness will suffice to initiate tumor rejection in individuals who are intrinsically tolerant to tumor antigens.

Proposed Course of Project:

The neuraminidase studies with lung tumors newly isolated from resistant strains should be emphasized in order to establish how generally the cross-reactive antigen is distributed. Extension of these studies to other tumors of other organ systems will then be attempted; these will be chosen to reflect a pattern of susceptibility and resistance among mouse strains which differs from the lung tumor pattern. The studies of cross-reactivity should be extended to determine the tissue localization and time of appearance of cross-reactive antigens during ontogeny. In collaboration with laboratories interested in immunotherapy, efforts will be made to exploit the cross-reactive immunity of tumor 85, in combination with non-specific immune stimulants, in an experimental immunotherapy system, first with "artificial" lung tumors induced by intravenous injection of tumor 85 cells, later with autochthonous chemically induced tumors. Careful attention will be paid to the possibility of autoimmune pulmonitis and other manifestations of autoimmune disease in such immunized mice.

Publications

Rice, J. M.: The biological behaviour of transplacentally induced tumours in mice. In Tomatis, L. and Mohr, U. (Eds.): Transplacental Carcinogenesis. IARC Scientific Publication No. 4. Lyon, France, IARC, 1973, pp. 71-83.

Martin, W. J., Esber, E., Cotton, W. G., and Rice, J. M.,: Derepression of alloantigens in malignancy. Evidence for tumour susceptibility alloantigens and for possible self-reactivity of lymphoid cells active in the microcytotoxicity assay. Brit. J. Cancer 28, Suppl. I: 48-61, 1973.

Serial No. NCI-4687
1. Experimental Pathology Branch
Carcinogenesis, DCCP
2. Perinatal Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Experimental Models of Human Childhood Neoplasms

Previous Serial Number: Same

Principal Investigator: Jerry M. Rice, Ph.D.

Other Investigators: William T. London, D.V.M., Donald M. Jerina, Ph.D., Russell W. Madison, D.V.M., and Wolfgang Wechsler, M.D.

Cooperating Units: NINDS, NIAMDD, Microbiological Associates, Inc., Cor-Bel Laboratories and Max-Planck-Institut für Hirnforschung, Cologne, Germany

Man Years:

Total	3.0
Professional	2.0
Other	1.0

Project Description

Objectives:

To develop experimental animal models for human cancers of childhood, and to use these models to study the greater sensitivity of fetal and neonatal animal tissues to chemical carcinogens, as well as the roles of factors other than the carcinogens themselves in enhancing or suppressing chemical carcinogenesis in experimental animals during intrauterine life and infancy. Carcinogenic organic compounds are synthesized where necessary; organic polymers are obtained commercially and characterized spectroscopically, and purified where necessary. Animals of various species and strains, including non-human primates, are exposed to these compounds at different times during pre- and postnatal life, and the carcinogenic response in various organs is evaluated histologically. Short and long-term cell and organ culture techniques are used where necessary to explore the role of interferon and other host defense mechanisms in resistance to carcinogenesis, and to study cocarcinogenic effects of microbial and viral infections in chemical carcinogenesis. Surgical procedures, including skin grafting, adult thymectomy, etc., are used in evaluating antitumor defense mechanisms of the intact animal, and organic and biological chemical techniques are employed to determine the effects of modifications of chemical carcinogen structure and of metabolic factors on the response of host tissues to carcinogens.

Major Findings:

In contrast to its predominantly neurotropic effects in rats, the carcinogen 1-ethyl-1-nitrosourea (ENU) induces chiefly epithelial tumors of the lung, liver, and other viscera when given transplacentally to mice. Approximately 100 neurogenic tumors have, however, been observed in ENU-treated mice (an incidence below 5% of animals at risk). These have been classified, and in some cases established as transplantable tumor and continuous cultured cell lines, in collaboration with Dr. Wolfgang Wechsler of the Max-Planck-Institut fur Hirnforschung, Cologne, Germany. This collaboration has been facilitated by Dr. Wechsler's visit to NIH in February, 1973, and Dr. Rice's visit to Cologne in October-November, 1973. Malignant neurinomas predominate among the tumors of mice as they do in rats, but in addition to the astrocytomas, oligodendrogliomas, ependymomas and mixed tumors common to both species, the mouse develops true cerebellar medulloblastomas and choroid plexus papillomas and carcinomas, which are not seen in the rat. These tumors occur in many strains of mice, especially strain C3H and its hybrids, as a consequence of exposure to ENU; the medulloblastomas especially are not restricted to the English strain in which they were first reported. The mouse thus appears the species of preference for studies on medulloblastoma.

Four of 7 transplantable neurinoma lines have been established in tissue culture, and all have been found to produce protein S-100, which is specific for the nervous system and provides a useful diagnostic confirmation of the origin of these tumors, which on histological grounds alone can easily be confused with other neoplasms. Efforts are in progress to extend cytologic ultrastructural and biochemical characterization to tumors of the glial, ependymal, and medulloblastoma series.

Transplacental carcinogenesis studies in the Old World monkey, Erythrocebus patas, have shown that pregnant animals and their fetuses tolerate up to 6 sequential intravenous injections of ENU, at a single-dose level of 0.1 mmole ENU/kg maternal body weight. This total dose is at least 10 times that necessary to induce a 100 per cent incidence of nerve tumors in rats. However, no tumors of any kind have yet been observed in any of 28 monkeys exposed to ENU in utero and observed up to 18 months after birth, or necropsied 1 to 14 months after birth following death from infectious disease.

The water-soluble polycation diethylaminoethyl (DEAE)-dextran has been found to be oncogenic in the rat and possibly in the Syrian hamster, after latency periods much longer than in the more susceptible mouse. Drs. H. Levy and S. Baron, NIAID, with whom collaborative studies on interferon inducers are performed, have shown that our complex of poly (I):poly (C) with the polycation poly-D-lysine is highly effective as an interferon inducer in primates, because of the protection afforded the polynucleotide against serum nucleases. In view of the potential use of this substance as an antiviral drug in man, the nature and limitations of the oncogenic activity of soluble polycatious are continuing under study. Leukemogenic viruses isolated from cultured cells of sarcomas induced by subcutaneous injection of such polymers have been shown to include agents having both B and N tropisms and

belonging to both Gross and FMR subgroups, but no focus-forming or sarcomagenic agent has been isolated. Studies are continuing on the possibility that the oncogenic activity of soluble polycations involves viral-chemical interaction.

Significance to Biomedical Research and the Program of the Institute:

The almost total lack of animal models of human pediatric neoplasms prevents laboratory study of factors affecting their development. The ability to induce medulloblastomas chemically in mice allows studies on the nature and inducibility of these tumors to proceed. The fact that such tumors are not induced in the closely related rat by identical treatment may provide an experimental approach to identifying factors which are important for their development. Most tumors induced transplacentally in rodents are of adult types and appear during adult life in individuals exposed in utero resembling human experience with diethylstilbestrol. The ENU studies in patas monkeys will provide experimental data indicating whether adult or pediatric tumor types are more likely to develop in at least one species of primate in response to carcinogenic exposure in utero, and thus whether chemical carcinogens are likely to be involved in the genesis of pediatric tumors in man. A new class of chemical carcinogens, the water-soluble organic polycations, have proposed uses in medicine such as inclusion in veterinary vaccines and in interferon-inducing complexes. Furthermore, they are non-reactive chemical carcinogens, differing markedly in this respect from the usual low molecular weight organic compounds, and elucidation of their mechanism of action, particularly resolution of the question of the possible role of oncogenic viruses, may provide a useful tool in the study of combined effects in chemical carcinogenesis, as well as provide a rational basis for their inclusion or exclusion from pharmaceutical use on the basis of carcinogenic activity.

Proposed Course of Project:

Transplantation studies of mouse neurogenic tumors, and biochemical and ultrastructural characterization of cloned cultured cells derived from these, would be continued with emphasis on medulloblastomas. The relationship, if any, of medulloblastoma cells to neuroblasts should be defined, and the propensity of the fetal mouse adrenal medulla to generate differentiated pheochromocytomas, rather than neuroblastomas, in response to transplacental ENU should be explored.

Patas monkeys exposed to ENU either transplacentally or directly (after birth) should continue to be observed for the development of tumors. Pharmacodynamic studies will be initiated to compare maternal-fetal distribution and tissue/organ localization of ENU in this species, versus the rat and mouse.

The C-type leukemogenic viruses of NIH General Purpose Swiss-Webster mice should be further characterized to define their role, if any, in the induction of soft-tissue sarcomas by water-soluble polycations. Carcinogenesis studies of these polymers should be continued to determine the basis for their oncogenic activity.

Honors and Awards

Visiting Fellow of the Max-Planck-Gesellschaft, Max-Planck-Institut für Hirnforschung, Cologne, Germany; Oct. 11 - Nov. 12, 1973.

Member, NIH Fogarty International Center Committee on the Prevention of Fetal and Perinatal Disease, 1972 - present.

Publications

Rice, J. M.: Spontaneous regression of autochthonous malignant lymphomas induced in Swiss and NZW mice by 1-ethyl-1-nitrosourea. In Gilbert, J. R. (Ed.): Conference on Immunology of Carcinogenesis. Natl. Cancer Inst. Monogr. 35. U.S. Dept. of Health, Education and Welfare, Public Health Service. Wash. D.C., U.S. Govt. Print. Off., 1972, pp. 197-209

Oesch, F., Jerima, D. M., Daly, G. W., and Rice, J. M.: Induction, activation, and inhibition of epoxide hydrases: an anomalous prevention of chlorobenzene-induced hepatotoxicity by an inhibitor of epoxide hydrase. Chem. Biol. Interact. 6, 189-202, 1973.

Worthington, M., Levy, H., and Rice, J.: Late therapy of an arbovirus encephalitis in mice with interferon and interferon stimulators. Proc. Soc. Exptl. Biol. Med. 143: 638-643, 1973.

Rice, J. M.: An overview of transplacental carcinogenesis. Teratology 8: 113-126, 1973.

SUMMARY REPORT

LUNG CANCER BRANCH

July 1, 1973 through June 30, 1974

"Plans, develops and implements a research program on the pathogenesis and prevention of lung cancer involving (1) identification and study of agents and mechanisms involved in the induction and development of lung cancer in man; (2) correlation of findings in human and animal studies in order to define relevance of animal models for study of human lung cancer; and (3) identification and study of agents and mechanisms capable of preventing the development of lung cancer. All these studies are pursued in the whole organism, in isolated cellular systems involving organ and tissue culture, and at the molecular level, in animals and man."

In 1970, a Lung Cancer Unit was established in the Office of the Associate Scientific Director for Carcinogenesis, to serve as an intramural focal point for the Special Lung Cancer Program. The program of the Unit has developed to the point that it has been established as a Branch in April, 1973, with two sections -- the Lung Cancer Pathogenesis Section and the Differentiation Control Section.

Current studies in the Branch include investigations on the carcinogenic effects of different chemicals on the respiratory epithelium in the whole animal, the use of short-term and long-term organ culture systems to study the effects of carcinogens and anti-carcinogens on respiratory epithelium, investigation of the molecular mechanism of action of lung carcinogens in cell-free systems, and investigation of the molecular mechanism of action of the anti-carcinogenic substance, vitamin A. Studies are pursued within the Branch in a manner that allows correlation of findings at the molecular, cellular, and whole animal level. The intramural program of the Lung Cancer Branch was developed along high priority program lines and is therefore closely coordinated with the contract program of the Lung Cancer Segment. Members of the staff provide scientific direction to the Lung Cancer Segment; in addition they contribute to the review and monitoring of some contracts in other segments. In this way, new information obtained at the basic level is rapidly disseminated and utilized in order to apply research results where they will ultimately be of most direct human benefit.

The following is a summary of the investigations within the Branch:

Lung Cancer Pathogenesis Section - *"(1) Develops and evaluates animal models for study of induction and prevention of lung cancer, and the role of various chemicals in inducing or preventing lung cancer. Studies involve the administration of carcinogenic and anti-carcinogenic agents to experimental animals and the evaluation of their effects on the development of lung cancer and (2) develops and evaluates organ culture and tissue culture methods for study of induction and prevention of lung cancer. Studies measure the effects of carcinogenic and anti-carcinogenic agents on the cell population which is directly involved in the development of lung cancer."*

(1) Animal Studies of Respiratory Carcinogenesis - Studies have been continued on the development of squamous cell carcinoma of the respiratory tract of the hamster, using both benzo[a]pyrene (BP) and N-nitroso-N-methyl urea (NMU) as carcinogens. It has been found within the past year that NMU can markedly potentiate the carcinogenic effects of BP on respiratory epithelium, particularly in terms of shortening the latency period for tumor development. These studies contribute to define maximal response conditions for induction of respiratory tumors in the experimental animal. Further electron microscopic studies have been performed on human biopsy material from patients with bronchogenic cancer or with preneoplastic lesions of the bronchi. These studies confirm that there is a meaningful correlation between the morphological findings seen in the animal model and in the human situation. Autoradiographic studies have been performed on the respiratory epithelium of the hamster after administration of BP or NMU and have shown that these carcinogens cause enhanced cellular proliferation. Animal studies on the effects of the potent and widespread human respiratory carcinogen, asbestos, have begun during the past year, but it is too early to report results. These studies will focus on the ability of asbestos to enhance cellular proliferation in respiratory epithelium, as well as its ability to act synergistically with BP.

(2) Studies on the Isolated Tracheal Epithelium of the Hamster - Short-Term Organ Culture Studies - Many new studies have continued to use the short-term tracheal organ culture method which has been developed in this Branch. Studies have continued on the binding of carcinogens, particularly BP, to epithelial cell DNA in this system. Correlations between the extent of binding of BP and its carcinogenic effects are being developed. Strains of hamsters with high susceptibility to carcinogenesis show higher binding of BP to DNA than that found in strains with low susceptibility. An enhanced binding of BP to DNA has also been found in vitamin A-deficient animals, which are believed to have an enhanced susceptibility to respiratory carcinogenesis. Conversely, inclusion of high levels of retinyl acetate in the organ culture medium inhibits binding of BP to DNA. The above studies are directed towards defining biochemical parameters which eventually may be useful in determining susceptibility of human populations to lung cancer. New studies on DNA repair in respiratory epithelium have also been started during the past year, using the short-term organ culture system. After exposure of tracheas to the carcinogen, NMU, it has been found that there is widespread low-level thymidine labelling of cells throughout the epithelium, indicative of the type of synthesis found during DNA repair. The ability to repair genetic damage represents a key defense mechanism against carcinogens, and this problem will be further investigated during the coming year.

(3) Studies on the Isolated Tracheal Epithelium of the Hamster and Man - Long-Term Organ Culture Studies - Excellent progress has been made in this area during the past 12 months. A year ago, optimal conditions for long-term organ culture of tracheal or bronchial material were still being investigated. During the past year, a chemically defined medium, supplemented with insulin and hydrocortisone, has been found to give excellent long-term survival of both hamster and human tracheobronchial

material, for 2 weeks or more. Using this system, it has been demonstrated that retinyl acetate and several of its analogs can reverse keratinizing, squamous metaplastic lesions of hamster tracheal epithelium. The finding that new analogs of retinyl acetate, with modifications in the ring portion of the molecule, are directly active on the tracheal epithelium is particularly important. This finding opens the way for synthesis of new compounds that may have greater pharmacological usefulness than vitamin A in man for prevention of lung cancer. Studies are currently in progress in the Lung Cancer Branch to evaluate the direct effects of retinyl acetate and its analogs on preneoplastic respiratory epithelium of hamsters, in order to determine if the progression of these lesions can be arrested or reversed in organ culture. A scientific collaboration has been developed with the Hoffmann-La Roche laboratories, which provide the new synthetic analogs for study.

The organ culture methodolgy described above is now also being applied to study human bronchial tissues. Human material has been found to be well maintained in vitro, using electron microscopic and autoradiographic techniques to monitor the cultures. It has already been found that human bronchial epithelium can activate benzo[a]pyrene to a form which is very tightly bound to epithelial cells.

(4) Studies on Cell Biology of Epidermis - Other epithelial cell systems may be used as screening models for the response of respiratory epithelium to carcinogens or anti-carcinogens. A small level of effort in the Lung Cancer Branch is being devoted to such research. In particular, an epidermal cell culture system is being used to assay biological activity of new vitamin A analogs. Significant data can be obtained in less than a week. Such cultures employ chemically defined medium, without serum. Addition of retinyl acetate to these cultures causes a significant increase in cellular RNA content, which is potentiated by insulin and hydrocortisone. Several new analogs of vitamin A are extremely active in this epidermal cell culture system. The activity of analogs, as measured in the epidermal cell culture system, correlates well with their activity in the tracheal organ culture system. These tissue culture studies will serve as a primary screen for evaluation of biological activity of new synthetic vitamin A analogs, as well as being useful for further elucidation of the mechanism of action of these compounds. Since the eventual use of vitamin A analogs for cancer prevention in man is currently under consideration, it is of utmost importance to have the most efficient primary screen for assessment of activity.

Differentiation Control Section - *"Studies molecular processes involved in control of cellular differentiation in respiratory epithelium as related to the induction and prevention of lung cancer. Particular emphasis is placed on the biochemical mechanisms whereby selected compounds inhibit or prevent the development of lung cancer."*

Since vitamin A is a required substance for normal differentiation of respiratory epithelium, and since it inhibits the effects of carcinogenic substances such as benzpyrene, both in vivo and in vitro, studies on the

mechanism of action of this substance are being actively pursued. An investigation of the respiratory tract of vitamin A-deficient rats and their pair-fed controls showed that the respiratory epithelium contains a glycoprotein dependent on vitamin A for its synthesis. Its chemical constitution was determined by GLC chromatography, and it was found to contain fucose, mannose, galactose, hexosamines, and N-acetyl neuraminic acid. The role of retinyl-phosphate-sugar esters in sugar transfer reactions has been further studied. The role of vitamin A in synthesis of a specific mannolipid in liver has been established. Two different mannolipids have been separated for the first time by thin layer chromatography. The working hypothesis is that vitamin A may be functioning as a lipid structural carrier for mannose residues which are to be incorporated into membrane glycoproteins. The influence of vitamin A on the synthesis of glycoproteins in epidermal cell cultures has also been studied, and an enhancement of incorporation of fucose and glucosamine into specific glycopeptides has been found. In all of the above studies, the effects of vitamin A appear to be highly specific for particular glycoprotein or glycopeptide fractions. The above studies provide an insight into the fundamental role of vitamin A in determining structure and function of particular proteins, especially those of the cell membrane.

Serial No. NCI 4798
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Differentiation Control Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Role of Vitamin A in Control of Epithelial Cell Differentiation and Carcinogenesis

Previous Serial Number: Same

Principal Investigators: Luigi M. De Luca, Ph.D., Robert M. Barr, Ph.D., Carol S. Silverman, M.S.

Other Investigators: George Wolf, Ph.D., Gloria Rosso, M.S., Fabrizio Bonanni, Ph.D., Stuart H. Yuspa, M.D., Jerrold M. Ward, M.D., P.R. Sundarensan, Ph.D.

Cooperating Units: Massachusetts Institute of Technology, Cambridge, Massachusetts; Experimental Pathology Branch, NCI; Laboratory of Lipid Research, St. Joseph Hospital, Lancaster, Pennsylvania

Man Years

Total	: 3
Professional	: 2
Other	: 1

Project Description

Objectives:

To study control mechanisms of mucous-squamous cell differentiation by vitamin A and chemical carcinogens in epithelial tissues.

Methods Employed:

Vitamin A deficient rats and hamsters and their pair-fed controls were used for studies of glycoprotein and glycophospholipid synthesis in tracheal epithelium and liver.

For vitamin A excess studies, the mouse epidermal cell culture developed by Dr. Stuart Yuspa of the Experimental Pathology Branch of NCI was used. In this system vitamin A causes the appearance of mucous granules which are otherwise absent in normal conditions of differentiation.

In addition to the usual techniques of isolation of glycoproteins and glycophospholipids, a new technique has been developed to isolate two mannophospholipids of the general structure prenyl-phosphate-mannose. The technique involves the use of thin layer chromatography on silica gel plates of the lipid extract in $CHCl_3$(60): CH_3OH(25): H_2O(4). In this system the two rat liver mannolipids have Rf 0.25 and 0.42. The 0.25 Rf material corresponds to the in vitro synthesized vitamin A mannophospholipid. Previous methods did not allow the isolation of these two products, with the same degree of reproducibility. This separation is crucial for studies of precursor product relationship, as explained below. Also it allows characterization of the in vivo synthesized product by NMR and MS.

Major Findings:

Vitamin A deficiency studies in rat tracheal epithelium. We have previously found that the synthesis of a fucose-glycopeptide from rat intestinal mucosa is greatly decreased in vitamin A deficiency parallel with a decrease in the number of goblet cells. Moreover, in vivo administration of the vitamin to depleted rats restored the synthesis of the glycopeptide and the number of goblet cells to normal within 10 to 18 hours. The fucose-glycopeptide was localized in goblet cells of the rat small intestinal epithelium by indirect immunofluorescence. We have also reported that the respiratory tract of the hamster needs vitamin A for the synthesis of a glycoprotein of different chemical composition than the rat intestinal compound. An investigation of the respiratory tract of A-deficient rats and their pair-fed controls clearly demonstrated that the rat respiratory epithelium contains a glycoprotein dependent on vitamin A for its synthesis. Its chemical composition was studied by GLC chromatography. It contains L-fucose (1), D-mannose (1), D-galactose (0.38), D-hexosamines (4.74), and D-N-Acetyl-neuraminic acid (3.5). It is not known whether this glycopeptide is a component of the membrane or a secreted glycoprotein. It is of interest that the synthesis of this glycoprotein alone was affected by vitamin A deficiency.

Vitamin A studies in liver. We have previously reported the in vitro involvement of retinol (vitamin A) in the synthesis of mammalian glycophospholipids consisting of a retinyl residue linked by a phosphodiester bond to a monosaccharide. We postulated that a phosphate ester of retinol would be an intermediate and therefore investigated the synthesis of mannosyl-retinyl-phosphates from retinyl-phosphate. We have synthesized for the first time [^{1}H] and [^{3}H] retinyl-phosphate and shown their enzymatic conversion to retinyl-phosphate-mannose.

Parallel to the above in vitro studies, in vivo studies were conducted to isolate and characterize retinyl-phosphate-mannose and other prenyl-phosphate-mannoses. The separation of the two main compounds, of Rf 0.25 and 0.42, was obtained by thin layer chromatography in $CHCl_3$(60): CH_3OH(25): H_2O(4). In this system the in vitro

synthesized retinyl-phosphate-mannose has Rf 0.25. The in vivo synthesis of two mannophospholipids was demonstrated unequivocally for the first time. One compound has Rf 0.25, the other Rf 0.42 in the rat and 0.33 in the hamster, indicating a shorter polyprenol in the hamster. Although the Rf 0.25 mannophospholipid displays all characteristics of the in vitro synthesized retinyl-phosphate-mannose, definite conclusions on its structure will be drawn from NMR and MS. These are being performed. Vitamin A deficiency specifically affects the 0.25 Rf mannophospholipid, again indicating a structural involvement of the vitamin. It is our working hypothesis that the vitamin may be functioning as a lipid structural carrier for certain mannose residues which will then be incorporated into membrane glycoproteins. Thus in A-deficiency, the incorporation of ^{14}C-mannose into liver membrane glycoproteins should be greatly decreased. A preliminary investigation in the hamster reveals that in vitamin A deficiency the incorporation of mannose is 10% that of the normal, after 20 minutes from the injection of the label.

Studies on altered glycoprotein synthesis in mouse epidermal cells treated with retinyl-acetate in vitro. These studies were conducted in collaboration with Dr. Stuart Yuspa of the Experimental Pathology Branch of the NCI. Mouse epidermal cells in monolayer cultures retain the characteristic squamous keratinizing phenotype. Vitamin A alters the phenotypic expression of these cells, from keratinizing into mucous secreting. It is of interest to mention that this change has never been induced in vivo, when the vitamin was given per os or intraperitoneally in the adult. The in vitro conditions allowed the proliferation of the Golgi apparatus and of the endoplasmic reticulum. The vitamin alters the normal biosynthesis of glycoproteins as studied by labeling with ^{3}H-fucose and ^{14}C-glucosamine. Epidermal glycoproteins were enzymatically digested to glycopeptides and separated on DEAE-Sephadex-A50 columns, using different concentrations of LiCl. There was a two-fold increase in the incorporation of ^{3}H-fucose and ^{14}C-glucosamine in the 0.2 LiCl fraction from cells treated for 3 weeks with 12.5 µg/ml retinyl-acetate and 1.25% DMSO, compared to DMSO controls. No changes were noted in other fractions. The glycopeptide from vitamin A treated cells isolated in 0.2 N LiCl had a higher MW than glycopeptides from the same fraction of control cells.

Vitamin A studies in the chemically induced adenocarcinoma of the intestine, previously project #NCI-4554, now incorporated in this project. These studies were conducted in collaboration with Dr. Jerrold Ward of the Carcinogen Screening Section, Experimental Pathology Branch, CG, DCCP, and Dr. P.P. Sundaresan, St. Joseph Hospital, Lancaster, Pa. Chemically induced adenocarcinomas of the small and large intestine of the rat are being compared with the normal intestinal mucosa. Most studies have, so far, been conducted in the small intestine.

It has been found that the amount of vitamin A per cell is about one-half in the adenocarcinoma, than in normal small intestine. However, the amount of protein/cell is also half in the tumor; i.e., the tumor tissue contains twice the number of cells/wet weight. Thus the total amount

of vitamin A per wet weight and per protein is the same in the two tissues. Preliminary studies on the mucous product are being conducted to study differences from the normal product of differentiation.

Significance to Biomedical Research and the Program of the Institute:

This project contributes to the identification of key control mechanisms in epithelial cell differentiation; it is a part of the lung cancer program addressed to the development of mechanisms of inhibition and control of respiratory carcinogenesis. The control of mucus cell differentiation by vitamin A is also of great interest in helping to understand the mechanism of carcinogenesis in epithelial tissues in general. The vitamin controls goblet cell formation and its absence leads to hyperplasia of basal cells, squamous metaplasia and keratinization: a sequence of events similar to those taking place in the respiratory epithelium after exposure to benzopyrene. The fucose-glycopeptide, shown to be present in the mucous structures of most epithelia can be used as a specific marker for differentiation responses in epithelial tissues.

Proposed Course of Project:

(1) To determine the structure of the vitamin A mannophospholipid by NMR and MS. (2) To study its specific role as precursorof membrane glycoproteins. (3) To study possible alterations of vitamin A dependent mechanisms of membrane growth in solid tumors and in transforming tissues.

Publications

Bonanni, F., De Luca, L.M.: Vitamin A-dependent fucose-glycopeptide from rat tracheal epithelium. Biochim. Biophys. Acta. (In Press).

Maestri, N., and De Luca, L.M.: Mannose transfer from mannolipid to endogenous acceptors in hamster liver. Biochm. Biophys. Res. Comm. 53: 1344-1349, 1973.

De Luca, L.M.: Commentary on a tissue specific factor inhibiting DNA synthesis in mouse epidermis. Forscher, B.K and Houck, J.C. (Eds.): Cahlones: Concepts and Current Researches. Natl. Cancer Inst. Monogr. 38. Wash. D.C., U.S. Govt. Print. Off., 1973, pp. 91-92.

De Luca, L.M., Yuspa, S.H.: Altered glycoprotein synthesis in mouse epidermal cells treated with retinyl-acetate in vitro. Exp. Cell Res. (In Press).

Serial No. NCI 4792
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Morphogenesis of Squamous Cell Carcinoma of the Respiratory Tract in Man and Animal Models

Previous Serial Number: Same

Principal Investigator: Curtis C. Harris, M.D.

Other Investigators: David G. Kaufman, M.D., Ph.D., Umberto Saffiotti, M.D., Michael B. Sporn, M.D., Hollis Boren, M.D., Frank Jackson, Maria Yamaguchi, and Joseph M. Smith

Cooperating Units: Ultrastructure Unit, Experimental Pathology Branch, CG; Department of Medicine, University of South Florida, Tampa, Florida.

Man Years:

Total	:	0.6
Professional	:	0.2
Other	:	0.4

Project Description

Objectives:

Previous investigations of the histogenesis of squamous cell carcinoma in man and animal models have shown that respiratory epithelium becomes hyperplastic, squamous metaplastic and finally neoplastic. These sequential changes in morphology may be better understood and defined by ultrastructural analysis.

Methods Employed:

The method of Saffiotti and co-workers is used to administer intratracheal instillations of benzo[a]pyrene (BP) carried on ferric oxide particles in hamsters. This treatment produces squamous metaplasia and squamous cell carcinoma in the respiratory epithelium of the Syrian golden hamster. Following single or multiple intratracheal instillations of (1) BP-ferric oxide; (2) pyrene-ferric oxide; (3) ferric oxide; or (4) saline, hamsters are sacrificed serially up to the time of gross tumor development.

Hamsters given intratracheal instillations of N-methyl-N-nitrosourea were also studied. Specific segments of the trachea and bronchi are processed for high-resolution light and electron microscopic study.

In patients, with suspected lung cancer, grossly normal and abnormal bronchial areas are biopsied using a fiber-optic bronchoscope. The biopsied tissue is examined as outlined above.

Major Findings:

Respiratory epithelium was examined by high-resolution light and electron microscopy in patients with suspected lung cancer and animal models of bronchogenic carcinoma. In human bronchial epithelium, atypical squamous metaplastic areas showed widened intercellular spaces, pleomorphic nuclei and abnormal basal laminae. Similar results were found in serial-sacrifice studies of the tracheobronchial epithelium in Syrian golden hamsters following ten intratracheal instillations of either benzo[a]pyrene-ferric oxide (5 mg each, 3 times per week) or N-methyl-N-nitrosourea (0.5 mg, weekly). In addition, atypical cilia were seen in cells adjacent to squamous metaplastic cells in the respiratory epithelium of man and the animal models. Intracellular, compound and intercellular cilia were found. The ultrastructure of squamous cell carcinoma, adenocarcinoma and anaplastic carcinoma of the lung in the animal models was similar to that of neoplastic tumors of the same histological types found in man. Our current data are consistent with the hypothesis that basal cell hyperplasia precedes squamous metaplasia, which can then be followed by squamous cell carcinoma.

Significance to Biomedical Research and the Program of the Institute:

The incidence of bronchogenic squamous cell carcinoma in man has shown a progressive increase during the past 5 decades. The pathogenesis of bronchogenic carcinoma is being studied in animal models and in man by several experimental approaches by members of the Lung Cancer Branch. This multi-faceted approach allows meaningful correlation of morphological and biochemical changes occurring during carcinogenesis. Our intramural investigations are closely coordinated with the activities of the Lung Cancer Segment.

Proposed Course of Project

Continuation of comparative studies between the morphological lesions found in man and those observed in animal models.

Publications

Harris, C.C., Kaufman, D.G., Sporn, M.B., and Saffiotti, U.: Histogenesis of squamous metaplasia and squamous cell carcinoma in an animal model. Cancer Chemother. Repts. 4: 43-54, 1973.

Harris, C.C.: The epidemiology of different histologic types of bronchogenic carcinoma. Cancer Chemother. Rep. 4: 59-61, 1973.

Port, C. Henry, M., Kaufman, D.G., Harris, C.C., and Ketels, K.: Acute changes in the surface morphology of hamster tracheobronchial epithelium following benzo[a]pyrene and ferric oxide administration. Cancer Res. 33: 2498-2506, 1973.

Harris, C.C., Kaufman, D.G., Sporn, M.B., Smith, J.M., Jackson, F., and Saffiotti, U.: Ultrastructural effects of N-methyl-N-nitrosourea on the tracheobronchial epithelium of the Syrian golden hamster. Int. J. Cancer 12: 259-269, 1973.

Harris, C.C.: Cause and prevention of lung cancer. Seminars in Oncology, Vol. 1, 1974 (In Press).

Harris, C.C., Kaufman, D.G., Jackson, F., Smith, J.M., Dedick, P., and Saffiotti, U.: Atypical cilia in the tracheobronchial epithelium of the hamster during respiratory carcinogenesis. J. Path. (In Press).

Serial No. NCI 4567
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Activation of Chemical Carcinogens by Human Bronchial Epithelium

Previous Serial Number: None

Principal Investigators: Curtis C. Harris, M.D., David G. Kaufman, M.D., Ph.D., Arthur L. Frank, M.D., Valerio M. Genta, M.D., Elizabeth McDowell, D.V.M., Ph.D., Lucy Barrett, Ph.D., Benjamin Trump, M.D.

Other Investigators: Frank Jackson and Maria Yamaguchi

Cooperating Units: Department of Pathology, University of Maryland School of Medicine; Ultrastructure Unit, Experimental Pathology Branch, CG, NCI.

Man Years:

Total	0.6
Professional	0.2
Other	0.4

Project Description

Objectives:

The enzymatic activation of chemical carcinogens into forms which tightly bind cellular macromolecules has not been directly measured in the bronchial epithelium of man. The initial aim of this project is to survey the ability of the human bronchial epithelium to activate several different classes of known and suspected chemical carcinogens.

Methods Employed:

Surgical specimens of human bronchus were maintained as 0.5 cm explants in 60 mm petri dishes containing 2 ml of CMRL 1066 medium with 1 µg/ml insulin, 0.1 µg/ml hydrocortisone, 0.1 or 0.5 µg/ml β-retinyl acetate, 100 units/ml penicillin G and 100 µg/ml streptomycin. In selected experiments, either serum or albumin was added. The cultures were kept

at 37°C in a gas-tight box, which was rocked at 10 cycles/min so that the explants were submerged 50% of the time. The medium was replaced and the cultures gassed with 5% CO_2, 50% N_2 and 45% O_2 every 2 days. Tight binding of ^{3}H-carcinogens in bronchial epithelial cells was measured by quantitative light microscopic autoradiography (Cancer Res. 33: 2842, 1973) and biochemical methods (Cancer Res. 33: 2837, 1973).

Major Findings:

Human respiratory epithelial cells can be maintained in organ culture for at least 6 weeks. These cells actively incorporated ^{3}H-leucine, ^{3}H-uridine and ^{3}H-thymidine into macromolecules as determined by autoradiography (ARG). Their ultrastructure was essentially unchanged when compared to bronchial epithelial cells examined before organ culture. The activation of chemicals potentially carcinogenic in the human respiratory tract is being studied. For example, 7 day-old bronchial cultures were incubated with 25 μCi/ml of ^{3}H-benzo[a]pyrene (BP; 25 Ci/mM) dissolved in dimethyl sulfoxide for 3 hr at either 37°C or 0°C. After incubation at 37°C, ^{3}H-BP was bound in mucous, basal, ciliated and squamous metaplastic cells; a 5-fold increase in bound ^{3}H-BP was observed when compared to binding in bronchial epithelial cells incubated at 0°C. Approximately 50% of the ARG grains overlayed nuclei. Biochemical studies to further define the activation and binding of chemical carcinogens to DNA and other macromolecules in human bronchial cells are in progress.

Significance to Biomedical Research and the Program of the Institute:

This project is one facet in a research program to develop an experimental system to study carcinogenesis in specific target tissues of man, e.g., human bronchial epithelium. Since many environmental chemicals are in an inert form (procarcinogen) and require enzymatic activiation to become carcinogenic, this in vitro system may allow the identification and study of these procarcinogens. The process of activation, its genetic basis (if any), its organotrophic specificity and the effects of inhibitors of activation (potential anticarcinogens) can be studied in human tissue.

Proposed Course of Project

Continuation of the studies outlined above.

Honors and Awards

Guest Lecturer, Radiological Society of North America, Annual Meeting, "Immunosuppressive Drugs in Man: Their Oncogenic Potential," November 25, 1973.

Guest Lecturer, Department of Pathology, George Washington School of Medicine, "Response of the Respiratory Epithelium to Injury and Carcinogens," February 5, 1973.

Guest Lecturer, International Workshop, Tobacco Smoke Effects on the Non-Smoker, sponsored by Geneva University, Bermuda, "Response of the Respiratory Epithelium of Man to Carcinogens," March 27, 1974.

Member, Lung Cancer Segment, CG, DCCP, NCI

Member, Working Party for Therapy of Lung Cancer, DCT, NCI

Publications

Harris, C.C., Kaufman, D.G., Sporn, M.B., Boren, H., Jackson, F., Smith,J., Pauley, J., Dedick, P., and Saffiotti, U.: Localization of ^{3}H-benzo[a]-pyrene and alterations in nuclear chromatin caused by benzo[a]pyrene-ferric oxide in the hamster respiratory epithelium. Cancer Res. 33: 2842-2848, 1973.

Kaufman, D.G., Genta, V.M., Harris, C.C., Smith, J., Sporn, M.B., and Saffiotti, U.: Binding of ^{3}H-benzo[a]pyrene to DNA from hamster tracheal epithelial cells. Cancer Res. 33: 2837-2841, 1973.

Genta, V.M., Kaufman, D.G., Harris, C.C., Smith, J.M., Sporn, M.B., and Saffiotti, U.: Vitamin A deficiency enhances the binding of benzo[a]-pyrene to tracheal epithelial DNA. Nature 247: 48-49, 1974.

Boren, H., Wright, E., and Harris, C.C.: Quantitative light microscopic autoradiography. In David Prescott (Ed.): Methods in Cell Biology, New York, Academic Press, Vol. 8, 1974, pp. 277-288.

Harris, C.: Immunosuppressive anticancer drugs in man: Their oncogenic potential. Radiology (In Press).

Kripke, M.L., Steinmuller, D., Harris, C.C., Lofgreen, P.E., and Eichwald, E.J.: Effects of chronic cyclophosphamide and methotrexate treatment on skin allograft survival in mice and rats. J. Nat. Cancer Inst. 51: 1355-1358, 1973.

Wenk, M.L., Reddy, J.K., and Harris, C.C.: Pancreatic regeneration in the guinea pig caused by ethionine. J. Nat. Cancer Inst. (In Press).

Yuspa, S., and Harris, C.C.: Altered differentiation of mouse epidermal cells treated with retinyl acetate in vitro. Exp. Cell. Res. (In Press).

Meadows, A.T., D'Angio, G.J., Evans, A.E., Harris, C.C., Miller, R.W., Mike, V: Oncogenesis and other late effects of cancer treatment in children: Report of a single hospital study. Radiology (In Press).

Serial No. NCI 4569
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Cell Proliferation in the Respiratory Epithelium

Previous Serial Number: None

Principal Investigators: Curtis Harris, M.D., and Hollis Boren, M.D.

Other Investigators: David G. Kaufman, M.D., Ph.D., Curtis Port, D.V.M., Ph.D., and Steve Springer, Frank Jackson, and Maria Yamaguchi

Cooperating Units: Department of Medicine, University of South Florida, Tampa, Florida; Division of Life Sciences, I.I.T.R.I., Chicago, Ill.; Microbiological Associates, Bethesda, Maryland; Ultrastructure Unit, Experimental Pathology Branch, CG, NCI

Man Years:

Total	:	0.6
Professional	:	0.2
Other	:	0.4

Project Description

Objectives:

The aim of the project is to measure alterations in cell proliferation caused by chemicals and co-carcinogens and carcinogens. The association, if any, between rate of cell proliferation in the respiratory epithelium and its susceptibility to the oncogenic effects of chemical carcinogens will be determined.

Methods Employed:

Labeling index following injection with ^{3}H-thymidine as determined by autoradiographic methods and mitotic index following injection with colchicine were used as measures of cell proliferation. Methods for exposure of hamsters to chemical carcinogens and induction of vitamin A deficiency have been previously published by our group. In selected experiments, regeneration of the tracheal epithelium was initiated by

causing mechanical traumatic injury to the tracheal epithelium with a blunt intratracheal cannula.

Major Findings:

The Syrian golden hamster is relatively insensitive to mitotic arrest caused by colchicine when compared to other animal species. Before measuring the mitotic index in vitamin A-deficient and carcinogen-treated hamsters, we determined the dose of colchicine, which arrested the maximal number of mitotic cells in metaphase. This dose of colchicine was 10 mg/kg in the esophageal epithelium, 100 mg/kg in the duodenal epithelium, and 500 mg/kg in the regenerating epithelium of the trachea. The sensitivity to colchicine was, therefore, organ-dependent. The number of mitotic cells arrested by colchicine was increased in the tracheal epithelium of vitamin A-deficient hamsters. Incorporation of ^{3}H-thymidine into mucous and basal cells was also augmented. Traumatic injury to the respiratory tract caused regeneration of the tracheal epithelium. The peak level of regeneration was 48 hr following injury.

Intratracheal instillation of either benzo[a]pyrene-Fe_2O_3 or N-methyl-N-nitrosourea caused an increase in cell proliferation in the respiratory epithelium approximately 1 week after their instillation.

Significance to Biomedical Research and the Program of the Institute:

An increased rate of cell proliferation may alter the susceptibility of the respiratory epithelium to chemical carcinogens. Although this is unproven in the respiratory epithelium, this type of enhancement has been observed in a variety of other tissues. The high incidence of tracheal tumors in the hamster model may be explained, in part, by the traumatic injury to the epithelium caused by the intratracheal cannula. The effect of vitamin A deficiency on respiratory carcinogenesis is uncertain, but it may be a state of increased susceptibility to respiratory carcinogens.

Proposed Course of Project

The effect on cell proliferation of chemical injury by HCL and respiratory infections by influenza virus will be studied. The proliferative response of the respiratory epithelium to chemical carcinogens will be further characterized. A serial-sacrifice study to determine if focal preneoplastic lesions in the hamster respiratory can be identified by high-resolution light microscopy and concomitant measurement of cell proliferation by autoradiography is in progress.

Publication

Harris, C.C., Silverman, T., Jackson, F., Smith, J., and Boren, H.: Proliferation of tracheal epithelial cells in normal and vitamin A deficient hamsters. J. Nat. Cancer Inst. 51: 1059-1062, 1973.

Serial No. NCI 4566
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lunc Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Susceptibility States and Human Lung Cancer

Previous Serial Number: None

Principal Investigator: Curtis C. Harris, M.D.

Other Investigators: Martin Cohen, M.D., Aron Primack, M.D., Richard Talamo, M.D., and Geno Saccomanno, M.D., Ph.D.

Cooperating Units: NCI-VA Oncology Branch, DCT, NCI; Department of Pediatrics, Harvard Medical School; St. Mary's Hospital, Grand Junction, Colorado.

Man Years:

Total	0.1
Professional	0.1
Other	0.0

Project Description

Objectives:

The purpose of the present study was to determine if individuals with lung cancer have an alpha$_1$-antitrypsin (AAT) deficiency, which could be a predisposing factor to the oncogenic effects of either cigarette smoke or other environmental carcinogens.

Methods Employed:

Clinical Material - Seventy-three patients with non-resectable and histologically proven lung cancer (70 male, 3 female) were studied at the Washington Veterans Administration Hospital. Their mean age was 56 years (range 40-83) and 72% of the patients were Caucasian. They had smoked cigarettes for an average of 30 pack years, i.e., average number of packages of cigarettes smoked per day multipled by the number of years of smoking was 30. Patients, without lung cancer, hospitalized at the same insitution served as controls. The control patients were matched according to age, sex, and smoking history to the 26 patients with squamous cell carcinoma of the lung.

AAT Levels - The serum was stored at -20° until radial immunodiffusion assay by the Mancini method. The accuracy of this assay is ± 10%. All serum specimens were coded and assayed in a blind fashion.

AAT Phenotypes - The phenotypes of AAT were determined on acid starch gel electrophoresis by a modified method of Fagerhol.

Major Findings:

Serum levels of AAT were significantly elevated in the four major cell types of lung cancer, when compared to control levels. While the highest level was found in patients with large cell anaplastic carcinoma, the levels were not significantly different between the four histological types of cancer. The mean AAT level in hospitalized patients without lung cancer, who served as controls, was the same as that found in the general population. Patients with primary cancer at other sites also had elevated levels. Thus,malignancy is one of several exogenous and endogenous factors that increase serum alpha$_1$-antitrypsin.

The phenotypes of AAT are under genetic control. They are not affected by exogeneous factors and may be a more informative guide to susceptibility. The determination of AAT phenotypes in lung cancer patients is in progress.

Significance to Biomedical Research and the Program of the Institute:

The identification of individuals in the population who are highly susceptible to the oncogenic effects of cigarette smoke may allow active intervention and prevention of lung cancer.

Proposed Course of Project

Continuation of studies outlined above.

Publication

Harris, C.C., Primack, A., and Cohen, M.: Alpha$_1$-antitrypsin levels in the serum of patients with lung cancer. Cancer (In Press).

Serial No. NCI 4568
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: DNA Repair Synthesis in the Respiratory Epithelium of Man and the Hamster

Previous Serial Number: None

Principal Investigators: Curtis Harris, M.D., David G. Kaufman, M.D., Ph.D., Hollis Boren, M.D., and Valerio M. Genta, M.D.

Other Investigators: Arthur L. Frank, M.D., Benjamin Trump, M.D., Frank Jackson, and Maria Yamaguchi

Cooperating Units: Department of Medicine, University of South Florida, Tampa, Florida; Ultrastructure Unit, Experimental Pathology Branch, CG, NCI; Department of Pathology, University of Maryland School of Medicine

Man Years:

Total	:	0.6
Professional	:	0.2
Other	:	0.4

Project Description

Objectives:

Somatic mutation following damage to DNA by chemical carcinogens is thought to be one mechanism causing neoplastic transformation. Inaccurate or incomplete DNA repair synthesis of damaged DNA could increase the likelihood of somatic mutation.The ability of the respiratory epithelium to repair DNA damaged by chemical carcinogens is unknown. The initial aim of this project is to measure DNA repair synthesis in respiratory epithelial cells.

Methods Employed:

Hamster tracheal epithelial cells were exposed to respiratory carcinogens either *in vivo* by intratracheal instillation or *in vitro* in a short-term organ culture system developed in the Lung Cancer Branch (*J. Natl. Cancer*

Inst. 49: 743, 1972). Following in vitro incubation of the tracheas with ^{3}H-thymidine for 1, 3, or 5 hours, DNA repair synthesis was assessed by quantitative light microscopic autoradiography.

Major Findings:

In one series of experiments, normal hamster tracheas were incubated in short-term organ culture for 1, 3, or 5 hours with 50 μCi/ml ^{3}H-thymidine and 0.5 mM or 2 mM N-methyl-N-nitrosourea (NMU). Autoradiography revealed that more than 90% of all types of epithelial cells were labeled, increasing with increased time or dose of NMU. Similar results were obtained when DNA in tracheal epithelial cells was damaged by ultraviolet light. These results suggest that the DNA damaged by respiratory carcinogens in vitro can be repaired. The fidelity of this repair is unknown.

If hamsters are given an intratracheal instillation of benzo[a]pyrene Fe_2O_3 and 2 days later their tracheas are incubated in vitro with ^{3}H-thymidine for 5 hr, focal areas of lightly labeled cells are found. Additional studies are in progress to determine if this finding represents DNA repair synthesis.

Significance to Biomedical Research and the Program of the Institute:

This project contributes to the Lung Cancer Program designed to study neoplastic transformation in target cells of the respiratory tract of man and experimental animals.

Proposed Course of Project

Several classes of ultimate carcinogens will be compared as to their ability to damage DNA and cause DNA repair synthesis in hamster tracheal epithelial cells. Similar experiments utilizing bronchial epithelial cells of man are in progress.

Serial No. NCI 4570
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Intranuclear Distribution of DNA Repair Synthesis and Ultrastructural Lesions in Human Diploid Cells Induced by Ultimate Chemical Carcinogens or Ultraviolet Light

Previous Serial Number: None

Principal Investigator: Curtis Harris, M.D.

Other Investigators: Michael Lieberman, M.D., Robert Conner, Ph.D., Frank Jackson and Maria Yamaguchi

Cooperating Units: Ultrastructure Unit and Digestive System Carcinogenesis Section, Experimental Pathology Branch, CG; Biometry Branch, Demography

Man Years:

Total	:	0.6
Professional	:	0.2
Other	:	0.4

Project Description

Objectives:

Chemical carcinogens and ultraviolet light damage nuclear DNA, which then may be repaired by enzymatic synthesis. The intranuclear distribution of DNA repair synthesis is unknown. The aim of this project is to determine this distribution as well as to examine the concurrent changes in nuclear ultrastructure.

Methods Employed:

Treatment of Cells. WI-38 human diploid fibroblasts were grown to confluence in diploid growth medium containing neomycin (73.5) mg/liter) and 10% fetal calf serum. These contact-inhibited cells were exposed to either: 1) NAAAF (1 or 10 μm) or 7-BrMeBA (100 μm) in redistilled dimethyl sulfoxide (final concentration 0.5%). NMU (0.2, 1 or 2 mM; 2) UVL (200 erg/sqmm) administered at a rate of 14 erg/mm2/sec from a low pressure mercury lamp; 3) control cultures received dimethyl sulfoxide alone. Fifteen minutes after treatment,

^{3}H-methyl TdR (10 μCi/ml; sp. activity 50 Ci/mmol; New England Nuclear, Boston, Mass.) was added. Hydroxyurea was not added to the culture medium.

Quantitation of Autoradiograms. In autoradiograms of 1 μ sections of cell pellets, the randomness of the intranuclear distribution of the autradiographic grains was investigated by two quantitative statistical approaches. The first considered randomness versus a general non-randomness while the second looked specifically at the difference in grain density between the inner and outer portion of the nucleus.

Major Findings:

Confluent, non-S phase WI-38 cells showed autoradiographic evidence of DNA repair synthesis following exposure to either N-methyl-N-nitrosourea (NMU), 7-bromomethylbenz[a]anthracene (7-BrMeBA), N-acetoxy-2-acetyl-aminofluorene (NAAAF) or ultraviolet light (UVL; 254 nm). Analysis of autoradiograms revealed that the intranuclear distribution of DNA repair in these cells were found to be non-random, i.e., did not fit a random model. We then examined the equality of the grain density near the nuclear envelope ("outer shell") versus the density of the central nuclear area ("inner core"). When the grain densities were corrected for the heterogeneous distribution of nuclear DNA, all cases, but the 1 μM NAAAF trial, had grain densities of the "inner core" significantly higher than the grain densities of the "outer shell." The DNA in the "inner core" is primarily euchromatic, while the DNA in the "outer shell" is primarily heterochromatic in WI-38 cells. No alterations in either the ultrastructure of heterochromatin or in its peripheral distribution were observed in cells undergoing DNA repair synthesis at doses of carcinogens examined. However, electron microscopy revealed segregation of nucleolar granules and fibrils in cells exposed to either NAAAF, UVL or 7-BrMeBA.

Significance to Biomedical Research and the Program of the Institute:

Chemical carcinogens and UVL damage DNA causing induction of DNA repair synthesis in many types of mammalian cells. The damage by chemical carcinogens is thought to be primarily due to their alkylating DNA. Inaccurate or incomplete DNA repair synthesis of damaged DNA could lead to neoplastic transformation. This project may aid in the understanding of DNA repair synthesis and neoplastic transoformation.

Proposed Course of Project

Additional biochemical studies and morphological studies using electron microscopic autoradiography are needed to clarify the differential damage and/or repair of heterochromatic versus euchromatic DNA. These studies are in progress.

Serial No. NCI-4551
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Eukaryotic DNA Synthesis and Carcinogenesis

Previous Serial Number: Same

Principal Investigators: David G. Kaufman, M.D., Ph.D., and Valerio M. Genta, M.D.

Other Investigators: Curtis C. Harris, M.D., and William K. Kaufman

Cooperating Units: C.W. Dingman, M.D., Nucleic Acids Section, Chemistry Branch, NCI; J.W. Grisham, M.D., University of North Carolina School of Medicine, Chapel Hill, N.C., and G.J. Todaro, M.D., Viral Leukemia and Lymphoma Branch, NCI.

Man Years:

Total	1.4
Professional	0.9
Other	0.5

Project Description

Objectives:

The aim of this project is to determine features of eukaryotic DNA synthesis and the control of this process. Knowledge of these processes would be valuable since abnormality of growth control appears to be a general property of malignant tissues. The acute and subacute effects of carcinogens on DNA synthesis and its control processes in normal cells is the ultimate objective of these studies.

Methods Employed:

Partial hepatic resection in rats has been employed to provide a partially synchronized cellular population of normal cells in vivo without use of chemical synchronization methods. Brief or prolonged intervals of labeling in vivo with thymidine-^{3}H (TdR-^{3}H) have been used to preferentially label nascent or non-nascent DNA. Nuclei have been isolated from liver cells by gentle methods and have either been used in in vitro DNA synthesis

assays or disrupted and further fractionated. Methods employed for fractionation of nuclear constituents and purified DNA, included isopycnic gradient centrifugation, hydroxyapatite chromatography, and agarose-acrylamide composite gel electrophoresis. Electron microscopy has been used to study DNA and nuclear constituents and sophisticated high resolution autoradiography techniques have been used to determine distribution of radioactivity.

Major Findings:

DNA has been synthesized in vitro in isolated rat liver nuclei. DNA repair was present in nuclei of both S-phase and non-replicating cells and was an ATP-independent processes in this system. In contrast, replicative DNA synthesis was dependent on ATP and demonstrable only in nuclei of cell populations containing S-phase cells. These two processes also had different properties in terms of the effects of substrates, inhibitors and co-factors. In a separate series of experiments, nuclei were isolated from liver following in vivo labeling with TdR-^{3}H, then sonicated, and fractionated by isopycnic gradient centrifugation. DNA in the gradients was concentrated in two bands: the heavy band contained about 85% of nuclear DNA whereas the light band contained only about 15%. In vivo labeling with TdR-^{3}H resulted in higher specific activity DNA in the light band than in the heavy band at short labeling intervals. Longer labeling times resulted in equalization of DNA specific activities in the two bands. Single-stranded, radioactively-labeled DNA was present only in the light band after short labeling intervals. In addition, replicative DNA synthetic activity with endogenous DNA template was found only in the light band, and replicative DNA polymerase was extractable exclusively from this fraction. Neither light nor heavy band fractions of normal liver nuclei contained detectable replicative DNA synthetic activity.

Significance to Biomedical Research and the Program of the Institute:

These studies contribute to the basic understanding of the processes of DNA replication and the repair of DNA damage. The control of DNA replication and cell proliferation, and the repair of carcinogen induced damage to DNA, are currently significant subjects for investigations directed toward elucidation of the basic mechanism of carcinogenesis. These studies have been conducted in tissues and systems in which they are most readily and easily investigated. These results and observations contribute to the part of the lung cancer program directed toward an understanding of metabolic processes in respiratory tract tissues. Comparable studies can then be designed for this target organ of high susceptibility. Thus, these basic experiments provide the basis for new studies to be conducted in respiratory carcinogenesis in this and future years.

Proposed Course of Project:

The present studies will be continued as described above.

Serial No. NCI-4697
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Experimental Respiratory Carcinogenesis

Previous Serial Number: Same

Principal Investigator: David G. Kaufman, M.D.

Other Investigators: Umberto Saffiotti, M.D., Joseph M. Smith, Curtis C. Harris, M.D., and Bruce Zwilling, Ph.D.

Cooperating Units: Dr. Russell Madison, and Steven Springer, Microbiological Associates, Inc.

Man Years:

Total	:	0.4
Professional	:	0.3
Other	:	0.1

Project Description

Objectives:

The aim of this project is to elucidate causative factors and pathogenetic mechanisms of respiratory tract cancer and to define biological models for the experimental study of bronchogenic carcinoma.

Methods Employed:

Carcinogens are administered to Syrian hamsters by intratracheal instillations: (a) Benzo[a]pyrene (BP) as a particulate suspension attached to particles of ferric oxide (Fe_2O_3); and (b) Nitrosomethylurea (NMU) or (c) β-Propiolactone (BPL) dissolved in buffered saline. Experimental groups are designed to consider following points: (1) the carcinogenicity of various single or multiple dose regimens of NMU in the respiratory tract, (2) the carcinogenicity of BPL in the respiratory tract, (3) the synergistic or co-carcinogenic effects of administration of NMU (or dilute HCl) in addition to BP plus Fe_2O_3, and (4) the effects of treatments with either vitamin A or Ribi Vaccine on groups of hamsters to which a respiratory-tract carcinogenesis regimen had been administered. Results are to be derived from analysis of the gross and microscopic lesions

observed at death in experimental animals.

Major Findings:

NMU alone, administered intratracheally, caused a low incidence of respiratory tract tumors for all doses and numbers of treatments tested. Respiratory tract tumors were primarily non-malignant papillary tumors of the trachea and major bronchi. Tumors of other organs were observed, with colon the most common site. Regimens of BPL alone, administered intratracheally have not produced respiratory tract tumors in experiments still in progress. Multiple intratracheal instillations of BP plus Fe_2O_3, followed by multiple doses of NMU, results in a synergistic increase in tumor response when compared with the sum of tumors with BP plus Fe_2O_3 alone plus NMU alone. Tumors resembled those produced by BP plus Fe_2O_3 alone. It has been demonstrated that hamsters injected intravenously with Ribi Vaccine (BCG cell-wall preparation) become immunized. Immunity is determined by swelling of foot-pads following injection of PPD into the foot-pad. Intratracheal administration of Ribi Vaccine was not an effective route of immunization.

Significance to Biomedical Research and the Program of the Institute:

These studies are part of the efforts aimed at defining factors leading to the development of bronchogenic carcinoma in animal systems under conditions that resemble those of human lung cancer development. Studies in progress have the following as objectives: (1) bioassay of two alkylating agents for their effectiveness as respiratory carcingens; effective water-soluble rapidly-degraded carcinogens would offer great advantages in biochemical studies and in long-term co-carcinogenesis studies, (2) development of a carcinogenic protocol which would induce respiratory tract tumors in high incidence and with short latency, and (3) determine whether either vitamin A or Ribi Vaccine can modify (reduce) the lung tumor incidence in hamsters treated with current respiratory carcinogenesis regimens. These studies coordinate with, or compliment studies in progress both intramurally and in the extramural contract program of the Lung Cancer Segment.

Proposed Course of Project:

It is proposed to continue long-term carcinogenesis studies in progress. In addition, new studies will be initiated to examine properties of carcinogens, states of susceptibility of experimental animals, and animal treatments which may modify the anticipated tumor response. These studies will coordinate with short-term *in vitro* experiments conducted in the Lung Cancer Branch and in life-time carcinogenesis experiments in the contract program of the Lung Cancer Segment.

Serial No. NCI-4571
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Studies on the Role of Asbestos in Causation of Lung Cancer

Previous Serial Number: None

Principal Investigators: Arthur Frank, M.D., Michael B. Sporn, M.D., Joseph M. Smith

Other Investigators: Curtis Harris, M.D., David Kaufman, M.D.

Cooperating Units: None

Man Years:

Total	1.8
Professional	1.3
Other	0.5

Project Description

Objectives:

This is a new project. Exposure of man to asbestos materials has been associated with increased incidence of cancer, particularly lung cancer, mesothelioma, and colon and rectal cancer. Little is known about the mechanism of asbestos on tissue, and subsequent cellular changes after exposure. The objective of this project is to define some of these mechanisms more clearly, both in the whole animal and in organ culture.

Methods Employed:

Attempts to elucidate basic cellular responses to asbestos have been started using a whole animal model as well as hamster tracheal organ cultures. Some work has been done with a small number of cultured human bronchial specimens. Long-term experiments have also been designed to try to better understand the possible co-carcinogenicity of asbestos and known chemical carcinogens.

Hamster tracheas can be cultured in the presence of asbestos without evidence of acute toxicity or immediate necrosis, using techniques developed in the Lung Cancer Branch. The tracheas are cultured in the following

medium: CMRL-1066 with the addition of glutamine, insulin, hydrocortisone, with the fresh addition of beta-retinyl acetate. The media is changed every 2 days, and the cultures are maintained in an air-tight box with 45% O_2, 50% nitrogen, and 5% CO_2. These boxes are then rocked so that the tissue spends equal amounts of time submerged and exposed to the gas.

Major Findings:

Since this is a new project, no definitive data are presently available. However, using the UICC Standard Reference Samples of amosite and chrysotile, studies have already been done to determine the amount of asbestos, in what volume of diluent, that can be tolerated by hamsters. It appears that amosite, which is not hydrophobic to the same extent as chrysotile, is tolerated in larger amounts; up to 5 mg. can be successfully given to animals at one time in a volume of 0.1 or 0.2 ml. saline. Great difficulty is encountered with injections over 1 mg. in 0.1 ml. with chrysotile. With larger amounts the animals rapidly develop a fatal pneumonitis.

It is also clear that asbestos can be added to tracheal organ cultures, and its effects on cell proliferation are currently being determined.

Significance to Biomedical Research and the Program of the Institute:

Elucidation of the mechanisms whereby asbestos causes cancer offers a logical means to prevent such carcinogenic action. The ability of vitamin A to block carcinogenicity of asbestos is already under preliminary experimental investigation.

Proposed Course of Project:

To continue as outlined above.

Serial No. NCI-4793
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: In Vitro Metabolic Studies in Isolated Hamster Respiratory Tract Tissue

Previous Serial Number: Same

Principal Investigators: David G. Kaufman, M.D., Ph.D., and Valerio M. Genta, M.D.

Other Investigators: Curtis C. Harris, M.D., William K. Kaufmann, and Michael B. Sporn, M.D.

Cooperating Units: None

Man Years:

Total	1.3
Professional	0.8
Other	0.5

Project Description

Objectives:

The general aim of this project is to examine metabolic processes in a respiratory epithelium. The specific current objective of these studies is to determine whether a short-term in vitro assay can be established which will have a high rate of correlation with the results of long-term carcinogenesis studies. It is hoped that such an assay might indicate situations affecting the susceptibility of the hamster respiratory tract to tumor induction and provide experiments with more rapid turnover than life-time studies.

Methods Employed:

Techniques have been developed for maintaining isolated hamster tracheas in vitro as organ cultures for at least 4 hours. Short period of maintenance were specifically chosen so that conditions in vitro reflect the in vivo state as well as possible. Previous studies have shown that radioisotopically labeled nucleic acid precursors added to the incubation medium could be recovered in well preserved high molecular weight RNA.

Major Findings:

The current series of studies have shown that tracheas incubated in vitro in medium with benzo[a]pyrene-^{3}H (BP-^{3}H) had BP-^{3}H bound to the purified DNA extracted from the epithelial cells and banded in cesium chloride gradients. Prior intratracheal treatment of hamsters with BP plus Fe_2O_3 in vivo resulted in increased in vitro binding of BP-^{3}H to tracheal DNA. This enhanced binding was inhibited by incubation in the presence of 7,8-benzoflavone; these results suggest that binding may be composed of two components, one inhibitable by 0° temperature but not by 7,8-benzoflavone, whereas the induceable component appears completely inhibitable by 7,8-benzoflavone. There was increased binding observed in the tracheas of vitamin A-deficient hamsters and in certain inbred hamster strains. Furthermore, there appeared to be an inverse relationship between hamster age and induceable binding levels. The question of whether these results are relevant to, or predictive of the results of long-term carcinogenesis studies, is being addressed by comparable long-term carcinogenesis studies in progress in the collaborative contract program and in the Lung Cancer Branch. In addition, preliminary studies have demonstrated that administration of carcinogens either intratracheally to hamsters in vivo or during incubation of isolated tracheas in vitro resulted in increased thymidine incorporation into tracheal epithelial DNA. Hydorxyurea has been shown in this system to inhibit DNA replication in normal tracheas, as well as in tracheas subjected to physical injury.

Significance to Biomedical Research and the Program of the Institute:

This project contributes to the part of the lung cancer program, designed to develop biologic models for the study of respiratory carcinogenesis, its causative factors and host control mechanisms. This assay system permits in vitro identification of critical biochemical and morphologic alterations which occur in the course of respiratory carcinogenesis induced in vivo. The methods developed permit examination of certain aspects of nuclear and cytoplasmic metabolism in viable respiratory epithelial cells. The effects of carcinogens administered in vitro can be examined biochemically. Furthermore, these methods will permit direct biochemical study of a respiratory epithelium during the course of in vivo carcinogenesis. This work coordinates with the in vivo carcinogenesis bioassays being conducted under contracts.

Proposed Course of Project

During the next year, it is proposed to continue to explore how a variety of in vivo co-carcinogenic or susceptibility factors influence the binding of BP-^{3}H to tracheal epithelial cell DNA during in vitro incubation of isolated tracheas with medium containing BP-^{3}H. These experiments will be designed to test the correlation of these in vitro studies with lifetime carcinogenesis studies conducted in whole animals. In addition, DNA repair in tracheal epithelial cells will be further studied and the feasibility of using this system as an in vitro assay (or combined in vivo exposure and in vitro assay) for respiratory tract carcinogens (mutagens) will be explored.

Publications

Kaufman, D.G., Genta, V., Harris, C.C., Smith, J.M., Sporn, M.B., and Saffiotti, U.: Binding of ^{3}H-labeled-benzo[a]pyrene to DNA in hamster tracheal epithelial cells. Cancer Res. 33: 2837-2841, 1973.

Genta, V., Kaufman, D.G., Harris, C.C., Smith,J.M., Sporn, M.B., and Saffiotti, U.: Vitamin A deficiency enhances the binding of benzo[a]-pyrene to tracheal epithelial DNA. Nature 247: 48-49, 1974.

Serial No. NCI-4549
1. Lung Cancer Branch, Carcinogenesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Organ Culture Studies on Tracheobronchial Epithelium

Previous Serial Number: Same

Principal Investigators: Gerald Clamon, M.D., Michael B. Sporn, M.D., Dianne Newton, Mary Baker, M.S., and Joseph M. Smith

Other Investigators: None

Cooperating Units: None

Man Years:

Total	:	2.9
Professional	:	1.4
Other	:	1.5

Project Description

Objectives:

The principal objective is to study the direct effects of carcinogens and anti-carcinogens on the cell population which is of immediate relevance for development of the most common forms of human lung cancer, namely respiratory epithelium.

Methods Employed:

Classical methods of organ culture are being used. Tracheas are being obtained from either normal animals, from vitamin A-deficient animals, or from animals treated with respiratory carcinogens.

Major Findings:

The optimal conditions for long-term organ culture of hamster tracheobronchial epithelium have been defined. Explants of trachea survive well for 2 weeks in an atmosphere of 50% oxygen, 5% CO_2, and 45% nitrogen, when kept on a rocker platform and bathed in medium CMRL-1066. It has been demonstrated that retinyl acetate and its analogs can reverse keratinizing,

squamous metaplastic lesions of hamster tracheal epithelium caused by vitamin A deficiency. Vitamin A analogs with modifications of the ring portion of the molecule have also been found to be active directly on tracheal epithelium.

Significance to Biomedical Research and the Program of the Institute:

The finding that new analogs of retinyl acetate, with modifications in the ring portion of the molecule, are directly active on the tracheal epithelium is particularly important. This finding opens the way for synthesis of new forms of vitamin A that may have greater pharmacological usefulness in man for prevention of lung cancer. Studies are currently in progress in the Lung Cancer Branch to evaluate the direct effects of retinyl acetate and its analogs on preneoplastic respiratory epithelium of hamsters, in order to determine if the progression of these lesions can be arrested or reversed in organ culture.

Proposed Course of Project:

To continue as outlined above.

Serial No. NCI 4553
1. Lung Cancer Branch, Carcinognesis, DCCP
2. Lung Cancer Pathogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1973 through June 30, 1974

Project Title: Effects of Vitamin A and Analogs on Cell Cultures of Epidermis

Previous Serial Number: Same

Principal Investigators: Michael B. Sporn, M.D., and Nancy Dunlop, M.S.

Other Investigators: None

Cooperating Units: Stuart Yuspa, M.D., Experimental Pathology Branch, CG, DCCP

Man Years

Total	:	1.3
Professional	:	0.3
Other	:	1.0

Project Description

Objectives:

The aim of the project is to use epidermal cell cultures as a primary screen for evaluation of biological activity of new synthetic vitamin A analogs, as well as for further elucidation of the mechanism of action of vitamin A.

Methods Employed:

The new methods developed by Yuspa and collaborators for culture of pure populations of mouse epidermal cells are being used. These have been modified in the Lung Cancer Branch so that it is now possible to work in serum-free media.

Major Findings:

Additions of retinyl acetate to these cultures caused a significant increase in cellular RNA content. Addition of insulin and hydrocortisone to the cultures potentiated the effect of retinyl acetate on cellular RNA content. Retinoic acid has been found to be even more potent than retinyl acetate in these cultures. Several compounds in which the ring structure has been modified, such as alpha-retinyl acetate, have also been found to be active.

Significance to Biomedical Research and the Program of the Institute:

Since the eventual use of vitamin A analogs for cancer prevention in man is currently under consideration, it is of utmost importance to have the most efficient primary screen for assessment of activity. This system will provide such a screen.

Proposed Course of Project:

To continue as outlined above.

Publication

Sporn, M.B., Dunlop, N.M., and Yuspa, S.H.: Retinyl acetate: Effect on cellular content of RNA in epidermis in cell culture in chemically defined medium. Science 182: 722-723, 1973.

IV. COLLABORATIVE PROGRAM SUMMARY REPORTS

SUMMARY REPORT

OFFICE OF THE ASSOCIATE DIRECTOR
FOR CARCINOGENESIS

July 1, 1973 through June 30, 1974

This Office is directly responsible for a group of collaborative projects which represent large multidisciplinary programs or which do not fit particularly well into any single segment.

They include the Carcinogenesis Program area of the NCI's Frederick Cancer Research Center (Litton Bionetics, Inc.); the interagency agreement with the AEC in support of the carcinogenesis program at the Oak Ridge National Laboratory and the carcinogenesis bioassay and research program at the Eppley Institute for Research in Cancer of the University of Nebraska.

Two projects are in the field of mechanisms of in vitro cell transformation, one on the "Development of a Tissue Culture Transformation System for Aromatic Amine Carcinogens" at Columbia University, and one on "Carcinogenesis and Control of the Cell Cycle" at the Salk Institute.

Two projects cover carcinogenesis studies on human populations, one on the "Etiology of Esophageal Cancer in the Caspian Littoral Area of Iran" supported in collaboration with the International Agency for Research on Cancer and the British Medical Research Council; the other is "A study of Oncogenesis and Other Late Effects of Cancer Therapy" through Memorial Hospital.

In each of these projects there is considerable participation of members of the staff from different branches and offices and segment advisory groups as well as ad hoc groups have provided peer review and advice.

CONTRACT NARRATIVES

OFFICE OF THE ASSOCIATE DIRECTOR
FOR CARCINOGENESIS

July 1, 1973 through June 30, 1974

AEC-NCI INTERAGENCY AGREEMENT, (Oak Ridge National Laboratory) (Y01-CP-40013)

Title: NCI-AEC Carcinogenesis Program

Contractor's Project Director: Dr. Francis T. Kenney

Project Officer (NCI): Dr. Allen H. Heim

Objectives: The broad objectives are as follows: (A) to carry out large scale co-carcinogenesis studies in exposure chambers with controlled atmospheres containing selected chemical agents to be tested for direct carcinogenic effects on respiratory tissues or for co-carcinogenic effects with known carcinogens, radiation, and viruses; (B) to bring to bear on problems of chemical carcinogenesis the diverse scientific talents of the staff of the ORNL's Biology Division, and the scientific and engineering expertise of other ORNL components. A major part of the funding is associated with the first objective.

Specific objectives are as follows: (1) to develop and define animal models for study of the pathogenesis of human lung cancer with emphasis on interaction of exogenous and endogenous factors, using inhalation exposures as well as other methods of respiratory carcinogenesis studies; (2) to investigate the possible relevance of N-nitroso compounds to cancer, involving (a) a study of the formation of nitroso compounds from amines and nitrite in chemical systems and in vivo, (b) development of analytical methods for nitrosatable amines and a survey of their distribution and, (c) studies of the mechanism of action of carcinogenic nitroso compounds by correlation of structure/activity relations with biochemical reactions involved in carcinogenesis; (3) to analyze the mechanisms for repair of damage to DNA by chemicals or irradiation in mammalian cells and the role of such damage and repair in carcinogenesis; (4) to correlate both the histopathological aspect of the systemic immune response and anatomic factors involving the progressive growth of chemically-induced tumors with functional immunologic factors directed against tumor growth.

As stated above, one of the major objectives is to incorporate the scientific talents of the ORNL staff into the Carcinogenesis Program. The degree to which this has been accomplished may best be realized by presenting the objectives, major findings and significance of each of the particular efforts.

Date Contract Initiated: January 14, 1963

Current Annual Level: $1,206,000

1. Respiratory Carcinogenesis

Specific Project Director: Dr. Paul Nettesheim

Objectives: To obtain information on the pathogenesis of human lung cancer by laboratory animal experimentation. To investigate the potential cocarcinogenic effects of irritant particulates, gaseous air contaminants, and microbial agents relevant to the respiratory tract. Particular emphasis is given to common air pollutants, such as iron oxide particles, and products of incomplete combustion such as nitrogen dioxide and aldehydes, which occur commonly as air pollutants and are also found in high concentration in tobacco smoke. To develop a better understanding of the morphogenesis of lung cancer on the histological and cytological level in order to enhance the diagnostic specificity for the detection of preneoplastic lesions and preinvasive carcinoma; to develop new experimental models that will facilitate such studies. To study the influence of Vitamin A on cell differentiation and cell proliferation of respiratory tract epithelium *in vitro*, and to investigate the potential anti-neoplastic effect of Vitamin A and its analogs *in vivo* and *in vitro*.

Major Findings: (1) The final evaluation of a chronic inhalation study with iron oxide shows a definite cocarcinogenic effect of these particles on the lung tumor induction with diethylnitrosamine.

(2) A study to test the potential cocarcinogenic effects of inhaled nitrogen dioxide, formaldehyde, and acrolein on polycyclic hydrocarbon carcinogenesis in the respiratory tract is underway. First results are expected soon.

(3) Comparison of epithelial lesions induced by carcinogenic, toxic, and infectious agents were made on the histologic, cytologic, and electron microscopic levels. Separation of carcinogen-induced lesions from those induced by other agents was possible in most cases by integrating multiple morphological parameters.

(4) An *in vitro* system was developed for maintenance of mucociliary epithelium for extended periods of time. The dependence of cell proliferation and cell differentiation in this organ culture system on different nutritional conditions is presently being studied.

(5) The inhibitory effects of retinyl acetate on early lung tumor development in rats was confirmed in a second study. Whether the progression of these early benign squamous cell tumors to invasive carcinoma can be inhibited by retinyl acetate is being investigated in a current experiment.

(6) No significant effect of Vitamin A on the growth of a transplantable squamous cell carcinoma has as yet been demonstrated.

Significance to Biomedical Research and the Program of the Institute: The inhalation studies with irritant particles and gases are important for the identification of the pollutants with cocarcinogenic activity for the respiratory tract. The morphogenesis studies are hoped to provide significant leads for improving the early diagnosis of lung cancer and for the identification of preneoplastic lesions. Various studies on the effects of Vitamin A and its analogs are expected to yield information concerning increased susceptibility to lung cancer induction in hypovitaminosis A, studies on inhibition of tumor induction and progression by Vitamin A hypervitaminosis, or potential significance for preventive therapy of preneoplastic lesions of the respiratory tract.

Proposed Course: The cocarcinogenesis studies will be continued to identify factors important for the induction of respiratory tract neoplasia, and to elucidate the mechanism of action of specific agents. Attempts will be made to study, in better defined experimental systems, the biological significance of carcinogen-induced lesions of the respiratory tract epithelium. This will require clear definition and staging of the various lesions. These studies will be performed *in vivo* and *in vitro*. The inhibitory effects of Vitamin A on tumor induction will be explored further *in vivo* and *in vitro* in an effort to find the most effective compound and mode of administration, and to determine whether Vitamin A influences initiation or progression.

2. Nitrosamine Carcinogenesis

Specific Project Director: Dr. W. Lijinsky

Objectives: To investigate the possible relevance of N-nitroso compounds to cancer, involving (1) a study of the formation of nitroso compounds from amines and nitrite in chemical systems and *in vivo*, (2) development of analytical methods for nitrosatable amines and a survey of their distribution and (3) studies of the mechanism of action of carcinogenic nitroso compounds by correlation of structure/activity relations with biochemical reactions involved in carcinogenesis.

Major Findings: (1) The extent of formation of N-nitroso compounds from nitrous acid and a number of secondary and tertiary amino compounds that are food constituents, drugs and agricultural chemicals has shown large differences, depending on chemical structure of the amine, particularly at low concentrations. Feeding tests of several of these compounds together with nitrite in rats are in progress, but only two of them are completed. The analgesic aminopyrine, together with nitrite, has given rise to 100% incidence of hemangioendothelial tumors in the liver after 1 year of feeding of 250 p.p.m. in drinking water. Most of these tumors metastasized to the lungs. No hepatocellular tumors were seen after treatment with aminopyrine + nitrite or after dimethylnitrosamine treatment of these rats: neither did liver cell damage by CCl_4 treatment, prior to dimethylnitrosamine, lead to formation of hepatocellular tumors.

(2) In collaboration with Dr. P. Nettesheim, a dose response study was made of tumorigenesis by nitrosoheptamethyleneimine given to rats by intragastric intubation or by subcutaneous injection. A high incidence

of squamous lung tumors and esophageal tumors was seen after oral treatment, declining with lower doses, but there were no lung tumors following subcutaneous treatment, even at higher doses than those given orally: instead there was a low incidence of tumors of the nose.

(3) A quite reliable analytical method for isolation identification and estimation of secondary amines has been developed and applied to several types of food and to tobacco smoke condensate; analysis for tertiary amines needs considerable improvement, especially in the area of quantitative recovery.

(4) A number of substituted nitrosopiperidines, dinitrosopiperazines and four nitrosotrialkylureas have been fed to rats at the same molar concentrations (within each group) in drinking water. The types of tumor produced by each type of compound tend to be the same, but there have been large differences in tumor incidence and latent period, depending on the structure of the compound. It appears that substitution on the carbon atom alpha to the amine nitrogen in the cyclic compounds greatly reduces the carcinogenicity, while substitution at other positions might enhance carcinogenicity. Nitrosomethyldiethylurea induced more than 50% incidence of CNS tumors after feeding in water, while nitrosotrimethylurea induced none. Nitrosomethyldodecylamine has given 100% incidence of bladder tumors after continous oral administration to rats. An extensive test of nitrosocarbaryl, the product of interaction of the insecticide carbaryl with nitrous acid, is in progress; this compound is a very potent mutagen in bacterial systems.

Significance to Biomedical Research and the Program of the Institute: The possibility that N-nitroso compounds might be formed in the gastrointestinal tract from ingested amines and nitrites makes this a significant potential carcinogenic hazard for man. Since most of the N-nitroso compounds tested have proved carcinogenic in one or more species, it is unlikely that man will be refractory to them. To assess their significance, more must be understood about the mechanism of formation of N-nitroso compounds from nitrite and amines of different structures to which man might be exposed. The N-nitroso compounds, through studies of their chemistry and of their distribution and metabolism, could help in the understanding of the mechanisms of chemical carcinogenesis.

Proposed Course: In addition to the program outlined above, a comparative study of distribution and metabolism of a few selected N-nitroso compounds in several organs of one or more species in which they have organ specific tumorigenic effects has been proposed.

3. Repair Mechanisms in Carcinogenesis

Specific Project Director: Dr. R.B. Setlow

Objectives: (A) To understand the role of DNA-repair mechanisms in determining the responses of various human cells to carcinogenic agents; (B) develop and use a model system that is capable of relating quantitatively UV and chemical changes in DNA to tumor induction.

Major Findings: (1) The capacity for DNA repair of UV-damage in 40 normal and repair variant humans has been measured. The distribution of repair capacity measured 20 hrs after UV damage is bimodal. A peak representing very low repair contains 10 XP individuals and no normals, whereas the normal peak contains only 1 XP, several heterozygotes, several familial polyposis, Bloom's syndrome and progeroid cells.

(2) The DNA of human cells synthesized shortly after UV-irradiation is in smaller than normal size segments. Upon incubation the shorter segments are elongated and joined by the insertion of exogenous nucleotides to form high molecular weight DNA as in nonirradiated cells. These joining processes occur in normal human cells as well as in XP cells. At 24 hours after UV-irradiation, the newly synthesized DNA is in normal size segments in normal human and in XP cells. Since the latter cells do not excise dimers from parental DNA, some new type of repair system--one that seems to permit the synthesis of proper-size DNA from a damaged template has been observed. Caffeine inhibits this repair system.

(3) DNA repair assays on normal cells treated with the nitroso derivative of carbaryl--a common pesticide--indicate an apparently intimate and long-lasting interaction with cellular DNA. The interaction is detected by the ability of the compound to cause alkali-labile bonds in DNA.

(4) An excellent correlation has been observed between the life-span of 7 species and the extent of DNA excision repair after UV-irradiation of their fibroblasts. Repair is proportional to log (life span).

(5) In four cell types (HeLa, the diploid strain HSWP, WI38, and the latter's SV40-transformed VA-13 2RA) the rate constant for K^+ turnover during growth has been found to be 0.95-1.05 hr^{-1}. Conditions have been determined in which the binding of ^3H-ouabain to HeLa cells parallels the inhibition of K transport by this glycoside. Assuming a one-to-one correspondence between ouabain-binding sites and transport enzymes, it has been calculated that in the steady state each site transports 40K ions/second and, when inhibited by the tightly bound ouabain, the sites are replaced in 3-4 hrs by a cycloheximide sensitive process.

Significance to Biomedical Research and the Program of the Institute: Chemically-induced cancers may develop as a result of failure of cellular mechanisms for repair of damage to DNA. This work provides knowledge of the cellular mechanisms operative in repair and may lead to measures for prevention of cancers induced by chemical or physical agents.

Proposed Course: This program will continue with emphasis upon (1) correlation of UV and chemically-induced changes in DNA with the induction of tumors, and (2) membrane changes associated with induction of tumors.

4. Immunology of Carcinogenesis

Specific Project Director: Dr. M. G. Hanna, Jr.

Objectives: The ultimate aim of these studies is to correlate histopathologic aspects of both the immune response and anatomic factors involving the progressive growth and metastasis of chemically induced primary and transplanted tumors, with functional immunologic factors directed against successful tumor growth. This information should provide insight into the role of the immune system as a host factor in establishment of both spontaneous and chemically induced neoplastic clones. It also should suggest rational approaches aimed at focusing immunity against the tumor, as well as prevention of metastasis.

It is clear that the host has the capacity to react immunologically to tumor specific antigens. In sharp contrast to the successful, progressive growth in situ of solid tumors, there exists concomitant immunity at other sites. A major factor contributing to the success of tumors is the inability of the host to focus its functional immune capacity on the neoplasm. This could result from immunologic blocking, either by anatomic factors at the site of tumor growth, or by competitive factors such as blocking humoral components. These factors, plus the fact that there are demonstrated immunosuppressive effects of chemical carcinogens, may account for the inability of the host to control autochthonous antigenic tumors. Thus, for one to understand the host-tumor interaction sufficiently to modify against success of tumor induction or tumor growth, it is necessary that investigations be carried out on the full complexity of the various components of the immune response, and the anatomical factors that limit their expression. This has been done in several animal models, with primary emphasis being nitrosomine- and hydrocarbon-induced primary carcinomas in hamsters, and a transplantable, chemically induced syngeneic hepatocarcinoma in guinea pigs.

Modulation of the major components of the immune system in favor of the host, such as enhancement of delayed hypersensitivity as well as non-specific resistance to isogenic or autochthonous tumors, is another approach to be incorporated in these studies. Studies are planned and designed to identify the BCG-mediated systemic, immunologic and anatomic alterations capable of promoting tumor regression.

Major Findings: A temporal relationship between depression of cell-mediated immunity and papilloma and carcinoma development during treatment with 7, 12-dimethylbenz(a)anthracene (DMBA) was studied. The test antigens used were non-tumor-related allografts and DMBA-pretreated isografts and autografts containing clones of neoplastic cells. 1) Allografts were invariably rejected by DMBA-pretreated recipients, and the allograft survival times increased as a function of tumor burden on animals receiving optimal carcinogen treatment 2) The survival of carcinogen-pretreated isografts (with early stages of tumor development) was found to depend on immunologic competence, as indicated by the prolongation of graft survival on recipients treated with antithymocyte serum. The survival of isografts also increased with the number of DMBA pretreatments and the tumor burden of recipients. 3) An association of tumor burden with depression of the immune response to non-tumor-related and

tumor-related antigens is interpreted to be the result of competition between the test antigens and the antigens of developing autochthonous tumors. This interpretation is supported by the findings that DMBA pretreatment of animals did not depress the immune reponse to autografts containing clones of neoplastic cells. 4) Because of the distinctive host response to autografts it is concluded that the available pool of immunologically uncommitted cells in chemical carcinogenesis systems may be the limiting factor in the response against alloantigens and the neoantigens present in isografts.

Histopathology of tumor regression after intralesional injection of Mycobacterium bovis: Regression of transplanted syngeneic tumors established in the skin of inbred guinea pigs and elimination of regional metastases are achieved after intralesional injection of Mycobacterium bovis strain Basillus Calmette-Guerin (BCG). Studies were performed to evaluate specific immunity to tumor cells and to BCG in this therapy model. The results demonstrate that the process of BCG-mediated regression of established tumors has both local and systemic features, both of which involve host-specific immunity: 1) A requirement for early sensitization to BCG antigens was demonstrated in guinea pigs whose peripheral and central lymphocyte compartments were depleted as a result of pretreatment with antilymphocyte serum. A correlation was shown between ability to develop delayed sensitivity to a purified protein derivative of BCG and tumor regression after injection of BCG into established tumors. 2) The development of specific tumor immunity was shown to be an aspect of this experimental model. Guinea pigs with established tumors develop humoral and cell-mediated immunity during the course of BCG-mediated tumor regression. 3) Antibody with specificity for line-10 tumor cells was demonstrated by the positive immunofluorescence and C1 fixation and transfer tests of plasma from BCG-treated tumor-bearing guinea pigs. Systemic cell-mediated immunity was demonstrated by the classic lymphocyte-mediated rejection of line-10 tumor challenge in guinea pigs that had undergone BCG-mediated regression of line-10 tumors.

Ultrastructural studies of histiocyte-tumor cell interactions during tumor regression after intralesional injection of BCG. An ultrastructural study of the site of a transplanted syngeneic hepatocarcinoma and its draining lymph nodes in inbred guinea pigs, in the presence and absence of BCG has been made. The Bacillus was injected into the growing intradermal tumor 7 days after transplantation, at which time it had metastasized to the first regional lymph node. Critical morphological similarities were found among activated histiocytes in the various reactive regions: infiltrating at the tumor site, autochthonous to the draining lymph node, migrating from the tumor site to the subcapsular marginal sinus of the draining lymph node, and infiltrating into the medullary sinuses of the lymph node. This observation indicates that these are all forms of a specific class of cell within the macrophage-histiocyte series. The cytopathic effect of these activated histiocytes on tumor cells is mediated by cell surface contact; sizeable areas of fusion of the cellular membranes of histiocytes and hepatocarcinoma cells have been observed. Thus, destruction of target cells by nonphagocytic mechanisms may be a phenomenon related to activated but not specifically sensitized cells of the macrophage-histiocyte series.

Significance to Biomedical Research and the Program of the Institute: This work provides an essential analysis at the histological and pathologic level of the role of the immune system in combating development and metastasis of chemically-induced tumors. It may lead to a more definitive mode of cancer therapy based upon fortification of the capacity to reject tumors by immunological mechanisms.

Proposed Course: The comparative histologic and functional immunologic studies of the various tumor models should provide information relative to the following aspects of tumor immunology: 1) The specificity of the immunologic reactivity of the central and peripheral lymphoid tissue prior to and during progressive tumor growth. More specifically, evidence can be obtained regarding the cooperative or antagonistic interactions between thymus, spleen, and bone marrow and draining peripheral lymphoid tissues. 2) The anatomic and physiologic implications within the systemic immune response which limit the ability of the hose to focus immunity to the tumor. 3) The anatomic limitations at the site of the tumor growth. 4) A localization of neoantigens in central and peripheral lymphoid tissue as the immunologic crossreactivity of these lymphoid components to the antigen. 5) The degree of cytotoxic lymphocyte and histiocyte production in the peripheral immune components during early stages of tumor growth, as well as factors in the serum which can block or mask this reactivity. 6) The mechanism of BCG-mediated tumor regression and its relationship to specific tumor immunity.

COLUMBIA UNIVERSITY (NO1-CP-23234)

Title: Development of a Tissue Culture Transformation System for Aromatic Amine Carcinogens

Contractor's Project Director: Dr. I. Bernard Weinstein

Project Officer (NCI): Dr.Stuart Yuspa

Objectives: Epithelial cell cultures from rat and human tissues are being established and utilized to analyze the mechanism of cell transformation by chemical carcinogens. Th se epithelial cell systems may also eventually provide a simple in vitro bioassay for chemical carcinogens. The possible significance of activation of latent viral genomes during chemical carcinogenesis is under investigation in rat hepatoma cell cultures.

Major Findings: Evidence has been obtained that cell cultures derived from chemically induced rat hepatomas synthesize A and C type RNA viral particles. Whereas six hepatoma cultures, and a spontaneously transformed normal liver cell line, were positive for these particles, five independently isolated epithelial cultures from normal adult rat liver were negative. Evidence was obtained that the viral particles in hepatoma cells are due to activation of latent viruses rather than in vitro contamination. Although the cultures established from normal liver are epithelial by several criteria, they lack hydrocortisone inducible tyrosine transaminase, perhaps due to reversion in culture to a more fetal pattern of gene expression.

Techniques for increasing the yield and purity of the rat hepatoma associated virus (RHAV) have been developed. It has been found that it contains an unusual 114S RNA and lacks the 70S RNA typical of other RNA tumor viruses. The 114S RNA is single stranded, contains poly A and is made up of subunits which are less than 35S. Extensive studies on the biologic activity of RHAV have failed to demonstrate transforming activity in culture, "helper" activity, or ability to replicate in foreign cells, suggesting that it may be a defective viral particle.

The first temperature sensitive mutants of chemically transformed epithelial cells has been successfully isolated. These have properties of tumor cells when grown in culture at 36° but adopt several properties of normal cells when grown at 40°C. These changes are reversible with a 1-2 day lag when the temperature is shifted either up or down. These mutants provide a valuable tool for analyzing biochemical properties specific to the normal or tumor phenotype in chemically transformed cells.

Several epithelial cultures have been established from human tumors and are being analyzed for the possible presence of viruses similar to those which we have detected in chemically induced rat tumors.

Significance to Biomedical Research and the Program of the Institute: Although more than 80% of human tumors arise from epithelial tissues, most of the previous tissue culture work related to chemical carcinogenesis (and to oncogenic viruses) has employed fibroblast or embryonic cell cultures. There is a paucity of cloned epithelial cell cultures which can be used for in vitro studies on chemical carcinogenesis. The development of such systems will facilitate studies on the mechanism of chemical carcinogenesis and will also provide rapid and simple bioassays for compounds which may be carcinogenic.

Both RNA and DNA viruses are being detected with increasing frequency in spontaneous, radiation and chemically-induced tumors. Detailed studies are required to determine whether or not these viruses play a causative role in the induction of these tumors, are cofactors in the transformation process, or are merely symptomatic of an aberration in the control of gene expression that characterizes neoplasia.

Proposed Course: (1) Additional epithelial cell cultures will be established and characterized from rat and human tissues, both normal and neoplastic. (2) Attempts will be made to increase the efficiency and diminish the lag which occurs in the in vitro transformation of epithelial cells by chemical carcinogens. (3) The rat hepatoma associated virus (RHAV) will be further characterized biochemically and in terms of biologic activity to determine its possible role in hepatocarcinogenesis. (4) Mutants that are temperature sensitive in the expression of transformation, which recently were obtained from chemically transformed cells, will be analyzed for biochemical parameters essential to the expression of the tumor phenotype.

Date Contract Initiated: April 1, 1972

Current Annual Level: $132,000

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP-02076)

Title: Etiology of Esophageal Cancer in the Caspian Littoral of Iran

Contractor's Project Director: Dr. J. Kmet

Project Officers (NCI): Dr. U. Saffiotti
Dr. M. Schneiderman

Objectives: The purpose of this study is to attempt to identify etiologic factors associated with the striking variation in esophageal cancer incidence along the Caspian Littoral of Iran. In this region the incidence changes by thirtyfold in women and by sixfold in men in areas having physical separations of less than 300 miles. Case control studies have indicated that alcohol and tobacco smoking are unlikely to be of etiological importance in this area. A sample of the population divided according to area of residence and cancer incidence is being studied in respect to (a) food and drink habits, (b) occupation, (c) personal habits, (d) deficiency and other disease patterns, and (e) a limited number of genetic markers.

It is believed that a study of these characteristics may help us to identify those factors which are relevant to the geographic variation in incidence.

Major Findings: The preliminary results of the first year's support by the National Cancer Institute (NCI) of this joint British Medical Research Council/ International Agency for Research on Cancer(IARC) Iran/NCI study of esophageal cancer in the Caspian Littoral are consistent with a multifactoral hypothesis for the etiology of esophageal cancer in this region consisting of (a) the presence of a carcinogen in the food or general environment (perhaps nitrosamines) combined with (b) a possible dietary deficiency (most likely Vitamins C, A or B2 together with a protein intake which may be deficient in some of the essential amino acids). This suggestion is tentative and additional work is necessary. Case control studies in the high and low incidence areas were suggested as the appropriate next step in attempting to confirm or deny this hypothesis.

Within Iran, the following suggestions for links with esophageal cancer require further investigation:

a. Search for nitrosamines and polycyclic hydrocarbons in bread, sheeps' milk and derivatives, weeds which contaminate food crops, wild plants, and fodder (particularly for sheep).
b. The use of special foods by segments of the population, such as, for example, pregnancy diets.
c. Evidence for reduced intake of essential nutrients (other than those covered above).
d. The presence of elevated levels of nitrate, nitrite, or metal ions in traditional drinking water supplies.
e. Possible genetic factors.
f. Types of fuel used in cooking.

Similarly the following items appear to have little or no relation to the incidence of esophageal cancer in Iran.

a. Opium consumption

b. Carpet making
c. Eating of salted or smoked fish
d. Ingestion of tannins from tea
e. Tea consumption in general, either as related to quantity consumed or temperature at which it is drunk
f. Consumption of specific medicinal plants
g. Contamination of foods during storage
h. Alcohol consumption, except possibly in the areas of low incidence
i Tobacco usage, except for NASS chewing by Turkoman men
j. Use of dyes (in the making of carpets or felts)
k. Methods of meat preservation

Significance to Biomedical Research and the Program of the Institute: Esophageal cancer is probably the best example available to us of a tumor which would appear to be causally related to environmental factors. If those factors can be identified and removed from the human exposure situation, we would have made a major advance in the prevention of a specific human cancer.

Proposed Course: It is not likely that any single national or international organization could successfully complete a study in depth of the environmental factors which may be related to esophageal cancer incidence in this part of the world. As a result this study is being conducted through a collaborative effort involving the Institute of Public Health Research, Tehran; the Food and Nutrition Institute, Tehran; IARC Regional Center, Tehran; and the IARC headquarters in Lyon. It is unclear at this time whether additional financial support from the NCI will be needed to continue the ongoing study.

It should be useful to extend these studies into the areas of the USSR that border on Iran, i.e. Soviet Azarbijan, the Turkoman Republic, Soviet Armenia, etc.

Date Contract Initiated: November 14, 1972

Current Annual Level: $60,000

LITTON BIONETICS, INC. (FREDERICK CANCER RESEARCH CENTER) (NO1-CN-23294)

Title: Operation and Maintenance of the NCI's Frederick Cancer Research Center

Contractor's Project Director: Dr. Robert E. Stevenson

Project Officer (NCI): Dr. William W. Payne

Carcinogenesis Coordinator (NCI): Dr. Richard R. Bates

At the NCI's Frederick Cancer Research Center (FCRC) there is a diverse program of research and resource production in collaboration with the various divisions of the National Cancer Institute. Approximately 40% of the program is supported by the Carcinogenesis Program. These activities are organized as separate projects, each of which is managed by a senior scientist on the contractor's staff and is monitored by a member of the NCI staff.

The individual projects are discussed below:

Project #7

Title: Laboratory Studies of Bowel Cancer Etiology

Contractor's Project Manager: Dr. Milton Slein

Project Monitor (NCI): Dr. Sidney Silverman

Objectives: The objectives of Project #7 have been to investigate the metabolism of bacteria indigenous to the human colon in order to determine their abilities to produce carcinogens, carcinogen precursors or carcinogens in vitro, and to identify such compounds chemically. From the results of epidemiological, clinical and laboratory studies by others, it was decided to initiate tests with tryptophan and steroids as putative agents of colonic cancer mediated by bacteria. An expansion of the program is being made to include morphological, biochemical and physiological studies of colonic tissue maintained in organ culture for the purpose of determining changes that occur during transformation.

Major Findings: Most of the research has been on the metabolism of tryptophan and its derivatives and of steroids by anaerobic bacteria found in the intestinal tract. Under anaerobic conditions, intestinal bacteria were found to metabolize tryptophan to tryptamine, indole and indoleacetic acid. Five-hydroxytryptamine was formed from added 5-hydroxytryptophan. No evidence has yet been found that kynurenine is formed from tryptophan under anaerobic conditions. Rats consuming a high meat diet were found to have ten times the level of tryptophanase activity in their fecal pellets and six times as much free tryptophan in the feces as rats on standard laboratory chow. The metabolism of steroids found in bile by anaerobic bacteria is complex. For example, after adding cholic acid to a culture of Peptostreptococcus species, 36 peaks were found by gas chromatographic analysis of metabolites. Enzymes for this metabolism are constitutive.

Significance to Biomedical Research and the Program of the Institute: There is epidemiologic evidence that a high incidence of colon cancer is associated with high meat diet. Since the epithelium of the colon is in constant contact with anaerobic bacteria, it seems likely that their metabolic activities may be of considerable significance in determining the levels and types of carcinogens to which the epithelium is exposed. This project examines the bacterial metabolism of classes of chemicals now considered to be among the potential substrates for carcinogen production.

Proposed Course: Assay systems are being developed to permit rapid screening of potential carcinogens from among the metabolites being studied. These include bacterial mutagenesis systems and the development of organ culture techniques for the mucosa of the colon. The metabolic studies with bacteria will continue; the short-term tests will be used to select metabolites for particularly intense investigation.

PROJECT #8

Title: Large Scale Bioassay

Contractor's Project Manager: Dr. Borge Ulland

Project Monitor (NCI): Dr. Norbert Page

Objectives: The primary objectives of the Large-Scale Bioassay Project are: To conduct screening studies of selected environmental chemicals to determine their carcinogenic potential in Fischer rats and $B_6C_3F_1$ mice; to improve the protocols under which these studies are conducted; and to provide pathology support for these studies, for other projects at FCRC, and for other collaborative research with other laboratories.

Major Findings: Forty-eight chemicals were selected for consideration for testing this year, though it is anticipated that no more than 30 will actually be started on chronic test. These chemicals consist mostly of pesticides and industrial chemicals, though some compounds have been included for investigations on chemical structure--carcinogen activity relationships. Seventeen chemicals are now on chronic study in rats and 11 mice. An additional 14 chemicals in rats and 20 in mice have either completed or are in the process of undergoing subchronic experiments for selection of dosage levels to be used in chronic studies. Previously, NCI protocols called for six to eight week subchronic studies for this purpose. As a result of observations on this contract, it has been determined that it is necessary to extend this period to three months in order to get adequate data. Several training programs are under way to qualify the staff for the types of activities required in this program. These include training of animal caretakers, histology technicians, and necropsy technicians.

Significance to Biomedical Research and the Program of the Institute: This project plays a major role in the NCI program to identify environmental chemicals which have the potential for causing cancer.

Proposed Course: This project will continue to investigate the carcinogenicity of environmental chemicals by standard NCI protocols. In addition, they will undertake investigations designed to improve the nature of these protocols and to provide more meaningful ways to extrapolate the results of animal experiments to the problem of human carcinogenicity.

PROJECT #9

Title: Preparation and Characterization of Carcinogens

Contractor's Project Manager: Dr. Walter Zielinski

Project Monitor (NCI): Dr. Marcia Litwack

Objectives: This project provides analytical and synthetic chemistry support to other projects at FCRC and to cancer research laboratories elsewhere throughout the world. New analytic techniques are developed as needed. The

bulk of the analytic activity is in relation to the bioassay program for which chemicals are procured, analyzed for purity, stability, and homogeneity within feed mixtures. Feed and bedding are analyzed for pesticide and mycotoxin residues. Crude chemicals are purified when necessary for program purposes. The analytic resources are also used for detecting and identifying metabolites of extrinsic chemicals or biologic materials. Carcinogens, their analogs, and metabolic products are synthesized when suitable materials cannot be obtained commercially. The project also maintains a chemical repository for storage and distribution of chemical carcinogens and their analogs for reference purposes. The metabolism of nitrosamines in relation to carcinogenesis is under investigation.

Major Findings: Purity-impurity analyses have been carried out for 23 chemicals under biologic study in the bioassay project. Similar studies have also been performed on 11 other chemicals being considered for bioassay. The observation of lack of homogeneity of some chemicals after mixing in the feed demonstrates the importance of developing improved blending protocols. Low levels of chlorinated hydrocarbons have been found in feed samples, but so far no aflatoxins have been detected in any feed sample. Gas-liquid chromatography (glc)/mass spectral analysis of bacterial metabolites of cholic acid demonstrated 36 peaks including the parent compound. These were classified as bile acid derivatives on the basis of glc retention and ms fragmentation patterns, but none of the spectra were consistent with those of reference standards. Several chemicals have been synthesized for investigations at FCRC and 1,1-bis (p-chlorophenyl) ethylene, a metabolite of DDT has been synthesized for investigations at the International Agency for Research on Cancer in Lyon, France. In an investigation of this interaction of DMN-3_H and DMN-d_6 with biologic macromolecules, it has been observed that methylation of the N-7 position of quanine from rat liver RNA and DNA is at a reduced level with the deuterated chemical.

Analytic chemistry activities include the following: (1) routine high resolution gas chromatography capability for detection and measurement of trace impurities, as exemplified by the analysis of a technical Perthane sample for 40 impurities with measurement sensitivity for less than 0.001% relative peak area; (2) high resolution routine gradient elution liquid chromatography for purity-impurity analyses; (3) routine quantitative fluorescence scanning techniques in the thin-layer chromatographic analysis of aflatoxins and metallo-organics, and preparative scale tlc analysis; (4) quantitative atomic absorption techniques for trace metal analysis in chemical and biological matrices; (5) definitive glc and solid probe mass spectral analysis using methane and isobutane chemical ionization as well as electron impact modes; (6) microsampling techniques for high resolution IR spectral generation; (7) routine C,H,N analysis, including sulfur and oxygen; (8) differential scanning calorimetry (dsc) for accurate phase transition data; (9) the installation of a data processor for automatic integration and processing of signals from gas, liquid, thin-layer chromatography and disc; and (10) the development of computer programs for glc and mass spectrometry.

A chemical repository is now operating from temporary facilities while permanent facilities are being designed.

Significance to Biomedical Research and the Program of the Institute: Adequate bioassay research can only be done with proper analytic chemistry backup for the identification of chemicals being administered, analysis of their purity, and indication of the length of time they are stable under conditions of storage and administration. This project provides that type of support. In addition, it is a central distribution point for the supply of reference standards on carcinogenic chemicals and their analogs for laboratories throughout the world. An expanding activity of this project is the synthesis of chemicals needed to study the metabolism of carcinogens, or the relationship between chemical structure and carcinogenic activity. It is anticipated that in the future the project will develop the capability for synthesizing radioisotopically labeled chemicals for laboratory investigation. This is a resource which is useful to the Carcinogenesis Program both at FCRC and elsewhere throughout the world.

Proposed Course: A major activity will continue to be in analytic chemistry both as support for Project #8 and also increasingly to investigate metabolites of interest to other laboratories in the Carcinogenesis Program. Synthetic activities will increase and the capability of synthesizing isotopically labeled chemicals will be developed. When the permanent facility for the chemical repository is established, this activity will expand to become a center for the collection and distribution of chemicals for bioassay for carcinogenicity in addition to its role in the distribution of reference standards.

PROJECT #10

Title: In Vitro Bioassay Methodology for Potential Carcinogens

Contractor's Project Manager: Dr. Roman Pienta

Project Monitor (NCI): Dr. Allen Heim

Objectives: The objective of this project is to develop standardized in vitro bioassay methods for detecting potential carcinogens which may present a hazard for man. Emphasis is placed on defining standard experimental conditions and end points which are reproducible, rapid, and valid for detecting potential human carcinogens.

Major Findings: Hamster embryo cells grown on an x-irradiated feeder layer have been transformed by polycyclic hydrocarbon carcinogens by methods similar to those described by Sachs and by DiPaolo. The ultimate criterion of malignancy is growth of a neoplasm after transplantation of carcinogen treated cells to a susceptible host. Among the techniques suggested for identification of transformation in vitro, growth of transformed cells in soft agar appears to be the most reliable, but additional research is needed on this problem. Investigation of conditions associated with susceptibility to transformation indicate that primary cultures of fresh cells are more susceptible than frozen primary cells or cells from subsequent passages and that cells from 13-day old embryos are more susceptible than those from 14-day old embryos. The optimum incubation time of primary cultures before treatment with carcinogens is two days. A number of human cell lines have been tested for susceptibility to transformation by SV-40 virus. A cell line showing maximum susceptibility has been selected for study of transformation by chemical carcinogens.

Significance to Biomedical Research and the Program of the Institute: Increasing numbers of chemicals are being introduced into use each year thereby increasing the number of potentially carcinogenic chemicals which should be tested. In vivo testing is costly and time consuming. Consequently, the development of a rapid, relatively inexpensive, reliable in vitro test for carcinogens would provide a system for prescribing chemicals to select those most in need of chronic testing in vivo. Use of the prescreen introduces a much needed method for improving the selection of chemicals entered into the costly in vivo bioassay system thereby improving the efficiency and overall effectiveness of the entire carcinogen detection system.

Proposed Course: Efforts will continue on identification of experimental conditions leading to maximum susceptibility to transformation by chemical carcinogens by both human cells and cells of experimental animals. Transformation by a broader range of classes of chemical carcinogens will be investigated and effort will be applied to coupling the in vitro system with suitable chemical metabolism systems to activate carcinogens which are not directly carcinogenic.

PROJECT #12

Title: Production of Inbred and Hybrid Laboratory Animal Strains

Contractor's Project Manager: Dr. Raymond Ediger

Project Monitor (NCI): Dr. Thomas Cameron

Objectives: To provide for production of inbred, hybrid and outbred laboratory rodents of various species and strains, in healthy condition and of known genetic background.

Major Findings: The animal farm provides high quality laboratory animals for a variety of programs within NCI. During the calendar year 1973, over 252,000 animals of 26 different species or strains were produced for use in cancer research programs throughout the country. Approximately 35% of the program is supported and utilized by Carcinogenesis-supported research. The bulk of the output for the Carcinogenesis Program is in Fischer/344 rats and B6C3F_1 hybrid mice, the animals used in the standard NCI bioassay protocol. This is the major source of these animals used in bioassay contracts throughout the country. Several other strains of mice, including the nude athymic mouse, are also produced as are three strains of guinea pigs and one of rabbit. In addition to the production of young adult animals, timed pregnancies, newborn, and aging animals are also supplied. The animal farm has been able to meet all needs for these strains from FCRC laboratories and also supply significant numbers of NIH intramural laboratories and NCI contracts.

Significance to Biomedical Research and the Program of the Institute: Biomedical research of the scope and the quality envisioned for FCRC must be solidly based upon a resource of various animal species of the highest quality of health and genetic integrity. This has been essentially accomplished

within this fiscal year. The FCRC Bioassay task, much of the NCI intramural research and some of the collaborative effort have been given continuity by their use of the animals produced by the FCRC animal farm with the cooperation and direction of the NIH in-house animal genetics unit. The previous animal supply for Carcinogenesis, via DCT contracts, has not been able to provide suitable animals in the quantity required for the Bioassay Program. Maintaining genetic consistency between animal colonies separated in distance and in time of establishment has been difficult. Establishing the FCRC colonies based upon the NIH breeding stock should allow increased confidence in genetic and microbial stability so that the results of these various bioresearch efforts can be compared to one another.

Proposed Course: This project will continue to supply most of the animals required for the Carcinogenesis Bioassay Program as well as significant numbers of animals for other intramural and collaborative carcinogenesis programs. Special emphasis will be placed on meeting the needs of all research projects at FCRC. Additional species and strain needs for this program will be explored and breeding started in the remaining animal farm buildings which are not presently active.

PROJECT #15

Title: Procedures for Safe Handling of Chemical Carcinogens

Contractor's Project Director: Mr. Everett Hanel (Acting Director)

Project Monitor (NCI): Dr. Larry Keefer

Objectives: The hazards associated with standard chemical laboratory procedures when working with chemical carcinogens will be evaluated. All available data bearing on this subject will be gathered and, where necessary, laboratory studies to assess risks will be carried out. All data will be studied and processed and protocols developed for use by workers handling carcinogens.

Major Findings: There is an active search under way to find a permanent director for this project. Meanwhile, visits are being made to various laboratories handling toxic or carcinogenic chemicals to obtain available information on the handling, use, storage, and disposal of hazardous chemicals. Support was provided to enable FCRC to receive, store, and ship those chemicals in the repository formerly held at Microbiological Associates, Inc. and now being held and shipped by Project #9. All chemicals used in the FCRC bioassay program and those chemicals being held in the present repository are the subject of a literature search to identify and characterize the compounds so that safe handling, storage, and disposal procedures can be listed.

Significance to Biomedical Research and the Program of the Institute: Numerous laboratories throughout the world are working with chemical carcinogens. Until now, there has not been a central laboratory for the purpose of developing and analyzing safety techniques for the protection of carcinogenesis research workers and the surrounding community from hazardous exposure to chemical carcinogens. This project is designed to meet this need.

Proposed Course: Plans are being developed for renovating a building to provide permanent laboratory and office space for this project. A permanent director will be hired. A project will begin by reviewing known approaches to carcinogenesis laboratory safety. Subsequently, necessary research projects will be undertaken.

PROJECT #16

Title: Chemical Carcinogenesis

Contractor's Project Director: Dr. John Landon

Project Monitor (NCI): Dr. Richard Bates

Objectives: This project covers performance of basic research pertaining to carcinogenesis by chemical and physical factors or the prevention of cancer by these agents. The research objectives reflect those areas of science investigation that require a special emphasis or expanded activities and that can maximally utilize FCRC facilities. Such areas may include studies in bioassay, biological models, immunology, molecular biology, and specific organ research.

Major Findings: During the past year research in this project has been devoted particularly to the study of the active principles and mechanisms by which BCG enhances immunologic rejection of tumors. To identify the components of BCG responsible for tumor cell killing, established tumor transplants were treated by intralesional injection of different mycobacterial fractions including cord factor (trehalose-6,6'-dimycolate), lipid-free cell wall preparation and a fraction derived from lipid-free cell wall preparation. A mixture of the lipid-free cell wall preparation and cord factor cured a greater proportion of guinea pigs than did the different mycobacterial fractions alone. Water soluble mycobacterial fractions were tested in C3H mice by mixing the fractions with 1038 fibrosarcoma cells and observing the effect on intradermal tumor growth. Preliminary experiments suggested that one water-soluble fraction was able to suppress tumor growth, a different water-soluble fraction was unable to inhibit growth of the tumor. Tumor growth was suppressed when 1038 fibrosarcoma cells were mixed with peritoneal cells from BCG-treated donors prior to intradermal injection. If tumor cells were injected directly into the peritoneum of BCG-treated mice, tumor growth was also suppressed. These results suggest that peritoneal cells from BCG-treated animals possess tumoricidal activity.

Significance to Biomedical Research and the Program of the Institute: BCG can interfere with induction of cancer in experimental animals by chemical carcinogens as well as resulting in the regression of tumors into which it is injected. Elucidation of the factors affecting this response should improve the usefulness of this technique.

Proposed Course: Research will continue on the study of lipids affecting the immunologic rejection of tumors. This project will be expanded to include research on carcinogenesis and hormone target organs. Particular emphasis will be placed on studying factors affecting the susceptibility or resistance of the mammary gland to carcinogenesis and on developing an experimental model for

studying prostate carcinogenesis. Some investigations will be initiated on studying the molecular alterations associated with the fundamental transformation from normal to malignancy.

Date Contract Initiated: June 26, 1972

Current Annual Level: $4,143,800

MEMORIAL HOSPITAL (NO1-CP-23286)

Title: A study of Oncogenesis and Other Late Effects of Cancer Therapy

Contractor's Project Director: Dr. Giulio J. D'Angio

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: (1) Determination of the "spontaneous" incidence of second primary tumors in patients with Wilms' tumor, neuroblastoma, and soft tissue sarcomas by chart reviews of short-term survivors; (2) Determination of the incidence of "induced" second primary tumors by chart reviews of long-term survivors. The appearance of tumor by type and number will be analy e with respect to several variables thought to be significant to detect correlations that might exist, if any; (3) The examination of long-term survivors, especially those considered at high risk as a result of the analysis in number 2 above, to detect malignant and possibly premalignant conditions.

Major Findings: (1) The pilot study at Children's Hospital of Philadelphia of long-term survivors at particular risk of developing organ damage, was completed. It revealed that physical examination alone is inadequate to detect organ malfunction. Many patients treated with radiation, with or without chemotherapy had some degree of renal impairment (10/50) and pulmonary dysfunction (9/50) without physical or roentgen findings. No specific association between second malignant neoplasms and organ damage was detected in this group of intensively treated survivors; (2) The hospital chart review of long-term survivors of three index tumors at three institutions had been begun during the previous year and was completed. Analysis of the data was made relating the occurrence of second malignant neoplasms and several variables. Children treated for malignant diseases with radiation therapy and actinomycin D (with or without chemotherapy) have a decreased relative risk (by one-fourth) of developing second malignant neoplasm compared to those children treated with radiation therapy alone. Although the group treated with actinomycin D had a shorter median follow-up period than the group not given the drug, these relative risks are maintained using a case-control method of analysis in which patients were matched according to years followed. No other variables, such as index, tumor, age, or stage at diagnosis were found to be associated with second cancers. Antifolates appeared to increase the risk of developing second neoplasms. Additional cases (now being collected) are required to confirm these findings; (3) No increase in second neoplasms was found to date in either women with choriocarcinoma or children with lymphoma successfully treated with methotrexate.

Significance to Biomedical Research and the Program of the Institute: Chemotherapy, combined with surgery and radiation for the treatment of children with Wilms' tumor and soft tissue sarcomas, has resulted in significantly improved cure rates of these diseases. It is essential that the risks inherent in these treatment regimens of both organ damage and carcinogenesis be understood by physicians who follow these children. Methods for periodic examinations have been delineated by the study. Knowledge of the etiologic mechanisms of oncogenesis will be expanded by collection of data regarding second malignant neoplasms in this group of treated survivors. Ascertaining the incidence of second cancers in patients not subjected to radiation ahd chemotherapy, in patients with congenital malformations and familial diseases, as well as that associated with specific treatments, is of concern.

Proposed Course: A method has been devised and relevant forms have been distributed to cooperating institutions treating pediatric institutions in the U.S.A. and Europe to ascertain the following: (1) The frequency of second malignant neoplasms in children under 16 years of age at diagnosis or any time thereafter. (2) The relationship of diagnosis, age, stage, treatment, length of follow-up to the recurrence of second malignant neoplasms.

These data, collected from the several participating institutions, will be analyzed and presented for discussions at a Seminar on the Late Effects of Successful Treatment of Cancer in Children and Young Adolescents to be convened in Genoa, Italy on October 19, 1974. The Seminar participants will include those who contribute the aforementioned data, and a small group of guests, carefully selected for their breadth of knowledge in bio-medical sciences.

Date Contract Initiated: June 26, 1972

Current Annual Level: $80,383

NEBRASKA, UNIVERSITY OF (Eppley Institute for Research in Cancer) (NO1-CP-33278)

Title: A Resource for Carcinogenesis Bioassays and Related Research

Contractor's Project Director: Dr. Philippe Shubik

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The bioassay of suspected chemical carcinogens and definition of the biological and chemical mechanisms of the carcinogenic process are two principal research aims under this contract.

Major Findings: 1. Bioassay--Presently, numerous compounds are being tested in several species. Compounds are selected for bioassay on the basis of their carcinogenic potential, as suggested by physical or chemical properties and structures, or by epidemiological findings which indicate environmental exposure to the compounds presents a high cancer risk. The mass distribution to, or use by, the public of chemical agents of suspected carcinogenicity is yet another consideration in the selection process. Once a compound is chosen,

an experimental design is drafted, based on protocols which consider present knowledge and the compound properties. The appropriate method of administering the compound is next determined, respective of its chemical and physical properties, the comparative response in various species, and its dose response parameters. A guiding principle of experimental design is the desire for maximum extrapolation of the analyzed data, resulting from each study, to man. A general policy of collaborative research prevails throughout, utilizing the expertise of chemists, biologists, pathologists, and biochemists in implementing portions of many experiments and in elucidating results.

Highlights of selected bioassay studies are as follows: (a) The use of substituted hydrazines is widespread in our environment since they are employed in industry, agriculture, and medicine. At present, there are 20 known hydrazines which induce tumors in laboratory animals. Of these, 7 were discovered by Eppley investigators. These synthetic chemicals pose a formidable environmental hazard to man. In the first stage, the aim has been to study the simplest types of hydrazines, i.e.; the mono- and dialkyl- derivatives. Several hydrazines were shown during the past year to induce tumors in mice. These compounds and the chief organs which each affected were:

1, 1-dimethylhydrazine (lungs, blood vessels, liver, and kidneys), ethylhydrazine HCl (lungs and blood vessels), n butylhydrazine HCl (lungs) and 1-carbamyl-2-phenylhydrazine (lungs).

(b) The carcinogenicity of the environmental hydrcarbon anthanthrene was proven on mouse skin.
(c) The possible carcinogenic effects of Na-cyclamate, Ca-cyclamate, and Na-saccharine were studied in Syrian golden hamsters. No carcinogenic effects were found in any of these cases. However, calcification of the blood vessels was found after treatment with all 3 substances. The more severe calcification was seen after calcium cyclamate administration and the more mild, after Na-saccharine treatment.

2. Research on the Mechanism of Carcinogenesis--Basic research in this segment of the program is conducted at the chemical and host levels to determine the biological mechanisms of the carcinogenic process and to define the factors which may control or alter the course of neoplastic growth. A variety of research approaches are employed. The molecular aspects of carcinogenesis, for example, are actively investigated through in vitro studies of both normal and chemically-transformed cells. Also, the anatomical and metabolic fate of chemical carcinogens is examined, and sites of elective localization in terms of tumorigenesis, relative to the various compounds, sought. Possible routes of detoxification and excretion are also explored. Identification of each carcinogen's most active form, characterization of hormonal or genetic host factors critical to carcinogenic responses, and response comparisons in different species are the other integral parts of this program.

Selective examples of research projects include the following: (a) Kinetics of nitrosation reactions should be known in order to evaluate the importance of the formation of N-nitroso compounds from environmental chemicals in vivo or during food storage. Accordingly, the kinetics for the nitrosation of 14 secondary amines and 13 N-alkylamides have been examined. For secondary

amines, the rate of nitrosation increased 200,000 times as the basicity of the amines decreased, approximately in agreement with the equation log k_2 = 7.1 -pKa (k_2 = pH-dependent rate constant, pKa = basicity of amine). Thus ease of nitrosation can generally be predicted from the basicity of the amine. For N-alkylamides and pH-dependent rate constant for nitrosation varied about 10,000 times, depending on the structure of the amide. Here, too, a structural study can now be used to predict ease of nitrosation. The highest rate constant was found for p-ethoxyphenylurea (dulcin), formerly used as a sweetening agent until it was identified as a carcinogen. Nitrosation of the analgesic tertian amine drug aminopyrine (AP) was found by W. Lijinsky (while at this Institute) to give dimethylnitrosamine (DMN) very readily. The kinetics of the reaction were examined and it was found that the rate constant for DMN formation was higher than that for any secondary amine previously examined, except N-methylaniline. DMN could be detected after reaction for only 5 seconds at 0^0C. AP is no longer widely used as a drug in the United States, but is still used in Europe. (b) It was previously suggested by this research group that ascorbic acid could be administered with nitrosatable drugs to inhibit in vivo nitrosation, since in vitro tests showed ascorbate competed effectively with most amines for nitrite (Mirvish, et al, Science, 177:65, 1972). Gallic acid (3,4,5-trihydroxybenzoid acid) and sodium sulfite have been found to be effective as ascorbic acid in inhibiting morpholine nitrosation. Thus, these compounds, if not toxic, could perhaps be used in place of ascorbate.

In a test of ascorbate's action, in vivo, Cardesa, Mirvish, and Shubik (Proc. Soc. Exp. Biol. Med. 145:145, 1974) showed that intragastric administration of ascorbate effectively prevented the acute liver toxicity of dimethylamine plus sodium nitrite given by mouth. The dose-response curve for DMN indicated that ascorbate inhibited DMN formation from dimethylamine plus nitrite by at least 75%. Ascorbate had no effect on the acute toxicity of preformed DMN, however.

(c) A study was made of the disappearance of sodium nitrite from the rat stomach after giving food containing added nitrite. Nitrite concentration in the contents of the acidic glandular part of the stomach (where most of the intragastric nitrosation presumably occurs) was 11% of that in the food, sufficient for nitrosation to take place in feeding experiments of amines and nitrite in rats and mice. The mean pH of the glandular stomach was 3.6, close to the pH maximum for nitrosation of amines. (d) To investigate whether or not β-oxidation is an essential step in the carcinogenesis of some nitrosamines, dipropylnitrosamine (DPN) and its metabolites due to β-oxidation (2-hydroxy-propyl-propyl-nitrosamine (2-HPPN), 2-oxy-propyl-propyl-nitrosamine (2-OPPN) and methylnitrosamine) were studied in Syrian golden hamsters. The results, obtained by administration of equivalent toxic doses of the compounds, revealed that all the metabolites are carcinogenic in hamsters. No marked differences were found between the pathological patterns and latency of tumors in the various organs and the multiplicity of neoplasms, as well as their distribution, varied. All the compounds induced respiratory tract tumors. DPN had the most pronounced effect and 2-HPPN, the least. The metabolites also induced liver tumors (DPN, however, produced none). Kidney tumors were induced by 2-OPPN. In view of the results, it seems possible that the target organ in carcinogenesis is influenced by variations in molecular patterns of the nitrosamines. (e) In

investigations on the mechanism of steroidal action on the metabolism, cytotoxicity and carcinogenicity of 7,12-dimethylbenz(a)anthracene (DMBA), it has been reported that a number of these compounds induce hepatic enzymes which metabolize polycyclic aromatic hydrocarbons (e.g., DMBA, benzo(a)pyrene) and inhibit adrenal necrosis elicited by DMBA (A. Somogyi, et al, Life Sci. 10, II: 1261 1971). In further studies, pregnenolone-16X-carbonitrile (PCN), the most potent steroidal enzyme inducer, influences the spectral properties and catalytic activity of the hemoproteinmiety of hepatic microsomal enzymes in a way different than both phenobarbital and 3-methylcholanthrene, the characteristic representatives of the 2 major groups of microsomal enzyme inducers (A.Y.H. Lu, et al, Arch. Biochem. Biophys., 152:457 1972). Furthermore, PCN protects rats against the acute toxicity of dimethylnitrosamine (A. Somogyi, et al, Nature, 237:61, 1972), while it greatly inhibits the in vitro N-dimethylation of this carcinogen.

In the current studies it is hoped to answer the following questions: (1) Would various steroids inhibit the mammary carcinogenicity of DMBA as well? If the answer is yes, is this inhibitory action proportionate to the extent of the enzyme inducibility of the individual steroidal compounds? (2) What are the properties of these enzymes (microsomal hemoprotein, NADPH-cytochrome c reductase and the catalytic activity) in the rat liver? (3) Would the steroids induce microsomal enzymes in species other than the rat, and is this inducibility confined only to the liver or does it extend also to the extrahepatic tissues, such as the mammary glands, adrenals, intestinal mucosa, lungs, and skin?

(f) Benzo(a)pyrene, activated by 1-electron oxidation with iodine or ferric chloride or by a presumable electrophilic oxygen obtained from ferric chloride and hydrogen peroxide, produces a carbonium ion at position 6. In analogy the charge localization of the carbonium ion at the 6-position, induced by hydroxylase enzymes either through 1-electron oxidation, by attach of active electrophilic oxygen at the 1- or 3-position or by both mechanisms, would represent the distinctive molecular characteristic of the hydrocarbon reactive intermediate responsible for its chemical reactivity to cellular nucleophiles and its carcinogenicity.

The charge localization in the radical cation of the potent carcinogens 7-methylbenz(a)anthracene (7-MBA), 7,12-dimethylbenz(a)anthracene (DMBA) and 3-methylcholanthrene (3-MC) was obtained by 1-electron oxidation with iodine. The reaction with nucleophilic pyridine produced 7-N-pyridiniummethylbenz(a)anthracene from 7-MBA, whereas 7-N-pyridiniummethyl-12-MBA, 12-N-pyridiniummethyl-7-MBA and 5-N-pyridinium-DMBA were obtained from DMBA. The same activation procedure of 3-MC yielded 1-N-pyridinium-3-MC. A similar activation in biological systems may be relevant to their chemical reactivity with cellular nucleophiles and logically account for their potent carcinogenicity.

(g) The specific deuteration of the 1-methylene group of 3-methylcholanthrene (3-MC) showed a decreased tumorigenic effect of the labeled compound with respect to 3-MC. This isotopic effect, capable of modifying the carcinogenic activity of 3-MC, provides clearcut biological evidence of the validity of a proposed mechanism in tumor initiation, which implies the binding of 3-MC to cellular targets through the 1-carbon atom.

(h) The mechanistic metabolic formation of the corresponding hydroxymethyl derivatives from 7-methylbenz(a)anthracene and 7,12-dimethylbenz(a)anthracene in rat liver microsomal preparations was investigated in 2 different sets of experiments using $H_2{}^{18}O$ and $^{18}O_2$, respectively. A positive isotopic incorporation was detected when both hydrocarbons were incubated with $^{18}O_2$, demonstrating that the metabolic product is formed by the oxygen of air activated by hydroxylase enzymes.

(i) The antituberculotic drug isoniazid undergoes a major metabolic change in man, during which part of the chemical is acetylated to yield 1-acetyl-2-isonicotinoylhydrazine (AINH). It is estimated that as much as 50-90% of the dose is excreted in acetylated form. In man and in the various animal species, INH metabolism follows different pathways, during which a half dozen metabolites are formed. With regard to tumor induction in mice, one metabolite,isonicotinic acid, was negative, while another, a hydrazine, was shown to be a tumor inducer. Our new observation proves the tumorigenicity of AINH for the first time. These studies in progress of several other metabolites aim toward elucidating the mode of actions of INH and its metabolites in the carcinogenic process.

(j) A study of possible species differences in the metabolism of DDT was continued in an attempt to explain the mouse's susceptibility to DDT hepatocarcinogenesis, in contrast to the hamster's insensitivity to this effect. The major polar metabolites in the urine of both species were conjugates of DDA with glucuronic acid, glycine, and alamine. A neutral metabolite, DDE, was found in very small amounts in mouse urine and increased markedly when mice were maintained in dietary DDT, but was not seen in hamsters. Also, when mice and hamsters were maintained on 250 ppm dietary DDT for 6 weeks, DDE residues in the mouse livers were much higher than in the hamster. It appears likely that preferential metabolic conversion of DDT to DDE in the mouse accounts for its sensitivity to liver tumor induction, since DDE is more potent in liver tumorigenesis in mice than is DDT.

The transforming ability of DDT and several metabolites in vitro is being studied in mouse embryo cells and the bacterial mutagenicity system. In both systems, DDT, DDA, and DDE appear inactive, while DDD and DDOH are most active. DDD does produce lung tumors in mice, suggesting that those in vitro systems may be good pre-screens only for certain types of tumorigenic compounds.

The biliary excretion of DDT and DDA in rats was studied in an attempt to explain the animals poor urinary excretion of DDT metabolites. DDT is slowly excreted in the bile, whereas DDA is rapidly quantitatively excreted and performs extensive biliary excretion. The poor slow excretion of DDT metabolites is probably caused by the compound's disposition in fat and other tissues.

Current experiments are designed to characterize other minor urinary metabolites of DDT in the mouse and the hamster, and to determine DDT's metabolic pathway in the tissues in vitro. The metabolic activation of DDT to compounds which could bind to protein and nucleic acids is also being studied.

(k) In vitro studies are also underway.

(1) Gangliosides of normal rat embryo cells and their transformants, 3-methylcholanthrene (MCA), Rauscher leukemia virus (RLV) and combined MCA-RLV, were investigated by ^{14}C-glucosamine incorporation. The transformants exhibited an increased uptake of the substrate, but a decreased incorporation into the gangliosides. A simplification of the ganglioside pattern was also observed in the transformants, (i.e. an accumulation of N-acetylneuraminyl-galactosyl-glucosylceramine (GM_3) concomitant with a decrease in the larger gangliosides). Differences between the effect of viral and chemical transformation on the ganglioside composition were observed and are currently being investigated.

The enzyme thymidylate synthetase was elevated 50-and 20-fold respectively, in the MCA and RLV transformants, compared to control cells. The enzyme was subject to post-confluence inhibition in the control cells, but not in the two transformants.

(2) Malignant transformation of mouse embryo cells has been accomplished with several polycyclic hydrocarbons. Caffeine inhibited transformation, while croton oil enhanced its frequency.

(3) Studies to investigate the correlation between mutagenesis in the bacterial system and transformation in the mouse embryo cell system using DDT and several metabolites were conducted. In both systems, DDD, DDMS, and DDOH were active, while DDT, DDE, and DDA were slightly active or inactive.

(4) Treatment of primary cultures of fetal rat lung cells with carcinogenic and non-carcinogenic polycyclic hydrocarbons indicated that carcinogenic compounds caused proliferation of epithelial cells into damaged areas of the flasks, while non-carcinogenic compounds had no effect. These epithelial cells could be subcultured and several lines have given carcinomas after injection into newborn rats.

(l) The morphological characteristics of malignant histiocytomas of Syrian golden hamsters were studied by light and transmission electron microscopy and histochemistry. Forty-three tumors were observed in the liver. In addition, tumor growth were found in 6 lungs, 2 lymph nodes, and 2 spleens. Histologically, they were malignant histiocytomas or Kupffer cell sarcomas. Ionized iron, fat, reticulum and polysaccharides, mucopolysaccharides, and glycotic fatty acids contents were studied histochemically in the hamster. Large amounts of ionized iron and reticulum were observed, while the other 2 substances exhibited lesser reaction. Transmission electron microscopic examination revealed a fine structure in well-developed lesions--the cell nuclei with chromatin arrangement, the cytoplasms with organelles, including the prominent lysosomes and lipid droplets, and the cell surfaces with the various projections.

The light, transmission, and scanning electron microscopic examination of the induced lung lesions of Swiss mice revealed adenomas composed of alveolar cells, type B. Ultrastructural characteristics in the cytoplasm included lamellar and osmiophilic bodies, membrane-bound lake-like structures, dense particles, and myelin figures. Free cell surface characteristics were described and illustrated.

Significance to Biomedical Research and the Program of the Institute: The Eppley Institute has provided over the years a considerable output of major research results in chemical carcinogenesis and represents a major national resource in which chemical, pathological, and biological aspects of carcinogenesis are studied in an integrated program.

Proposed Course: This contract will continue to support the study, improvement and conduct of bioassay techniques in carcinogenesis. The project will continue as outlined in the objectives above.

Date Contract Initiated: March 19, 1968

Current Annual Level: $2,297,334

SALK INSTITUTE (NO1-CP-33405)

Title: Carcinogenesis and Control of the Cell Cycle

Contractor's Project Director: Dr. Robert W. Holley

Project Officer (NCI): Dr. Andrew Peacock

Objectives: The growth of normal and tumor cells is controlled in part by the genome of the cell and in part by extracellular agents. In addition to the supply of common nutrients (e.g. amino acids, sugars, etc.) the extracellular fluid contains hormones and analogous control substances, some of which influence the availability of the nutrients to the cell. Carcinogenesis changes the relationship between the cell and the extracellular agents such that growth rates greater than normal occur. However, it has been found that some regulations of growth still remain in chemically-induced tumor cells and the residual controls in these malignant cells is the subject of the present study. The overall objective of this study is to determine the kinds of changes that accompany chemical transformation of cells. The present project contributes to that goal, but focuses on mechanisms whereby the growth rate in chemically-induced tumors might be checked.

Major Findings: Some chemically-induced tumor cells can be arrested readily in the early G_1 (or G_0) phase of the cell cycle by limitation of serum. One such cell, a mouse myeloma, XS63·5, is dependent on a serum lipid, and growth is arrested when the lipid is depleted. Fractionation studies suggested that the active lipid was neutral and possibly a monoglyceride. It has now been found that the lipid can be replaced by an unsaturated fatty acid: oleic acid, linoleic acid or arachidonic acid. As far as can be determined, this is the first demonstration that a lipid can control the initiation of DNA synthesis and growth.

Significance to Biomedical Research and the Program of the Institute: This contract will attempt to provide more detailed information regarding the relationship between extracellular factors, membrane controls and intracellular factors in normal and chemically-transformed cells. This can provide insight into the rates of growth of different types of cancers which can serve as a basis of considering new means of controlling malignant growth.

Proposed Course: Growth control mechanisms will be studied in various chemically-induced tumor cells and in cells transformed chemically in vitro. From past experience, it is anticipated that some of these controls will involve previously unrecognized components of serum and attempts will be made to identify these components. Attempts to identify other growth regulatory mechanisms in chemically-transformed cells will involve manipulation of the composition of the medium as well as manipulation of serum fractions. There will be a search for interactions between different mechanisms.

Date Contract Initiated: June 26, 1972

Current Annual Level: $145,000

SUMMARY REPORT

BIOASSAY OPERATIONS SEGMENT

July 1, 1973 through June 30, 1974

Historical Development

The Bioassay Operations Segment evolved from an ongoing program, of more than ten years duration, to identify and evaluate carcinogenic hazards in the human environment. As a result of growing evidence that most human cancers are attributable to environmental factors, today the Bioassay Program has not only national, but worldwide importance. The Program is heavily involved in the testing of chemical and physical agents which may have great social, political and economic impact. Senior scientists working in the Program are frequently asked to proffer advice, at both the national and international levels, as to the carcinogenicity of chemicals tested by Bioassay Operations Segment contractors. In addition, the Program is often approached by industry and other governmental agencies for guidance as to proper bioassay procedures.

The rapid growth of the Program necessitated in 1971 the formation of a specific Bioassay Segment to administer, monitor, and develop its activities. As the Program continued to expand under the direction of Drs. John and Elizabeth Weisburger, the need for greater support capabilities and inhouse personnel to coordinate them became apparent. In response, the Information and Resources Segment developed resource contracts in the animal, chemical and data areas. To centralize the management and coordination of the resource and bioassay contracts, a reorganization within the Collaborative Contract Program resulted in the formation of the Bioassay Operations Segment in March, 1973. This Segment incorporated the standardized testing contracts from the abolished Bioassay Segment and the resource contracts from the Information and Resources Segment. In addition, during the past year the Bioassay Operations Segment has also become responsible for several contracts transferred in from other Segments.

In total expenditures, the Bioassay Operations Segment continues to be the largest in the Carcinogenesis Program, with an annual operating budget of approximately $7.5 million. This level, however, was not entirely reflected in FY-73's actual funding obligations. In FY-72, five major contracts were awarded and funded for initial 18 month periods. In addition, several short-term renewals were made during FY-73. These actions resulted in the Bioassay Operations Segment's FY-73 budget being considerably less than its actual operating level. Although the bioassay of many chemicals were completed during FY-74, a 1 to 1 replacement for them was not possible because of the unavailability of funds.

Segment Structure

As Director of the Bioassay Operations Segment, Dr. Norbert P. Page is responsible for the direction, implementation, conduct and administration of the Segment's program. Overall coordination of the Segment's activities is the

responsibility of its Manager, Dr. James M. Sontag. Because of its nature, the Bioassay Operations Segment requires expertise in animal husbandry and laboratory medicine, analytical chemistry, toxicology, carcinogen bioassay, pathology, and biometry as well as many related disciplines. Input into these areas comes from two sources. An Advisory Group, comprised of both government and non-government scientists, evaluates ongoing and proposed projects as well as recommends new program areas. A large amount of expertise is also provided by members of the Carcinogenesis staff acting as Project Officers.

Besides the Bioassay Operations Segment staff, the Advisory Group, and Project Officers, the success of the Program is largely dependent upon the contributions of other carcinogenesis staff members. The judicious selection of test chemicals is the responsibility of the Chemical Selection Committee, chaired by Dr. Elizabeth Weisburger. Chemicals are choosen primarily on the basis of human exposure, production data, and structure-activity relationships. Dr. Marcia Litwack, Executive Secretary of the Committee, coordinates the chemical resources responsible for the procurement, analyses, and distribution of the test agents. The timely delivery of high quality animals of known genetic background and in sufficient quantities is the responsibility of Dr. Thomas Cameron, who coordinates animal resources. In the past, animals have been procured primarily through suppliers developed by the Division of Cancer Therapy. During the last year, however, SPF colonies at the NCI Frederick Cancer Research Center's Animal Farm were sufficiently developed to take over this activity. Each of the breeding colonies were started with stocks supplied by the NIH Veterinary Resources Branch. The staff responsible for the Carcinogenesis Bioassay Data System, described in another section of this summary report, is concerned with the assemblage of data in a manageable and retrievable form. In addition to these relationships, close communication is maintained between the Coordinator for Environmental Carcinogenesis, Dr. Herman Kraybill; the Head of the Tumor Pathology Section, Dr. Robert Squire; and with members of the Carcinogen Metabolism and Toxicology, and Information and Resources Segments.

Plan of Operation

The primary goal of the Bioassay Operations Segment remains the same today as when the large-scale testing program was conceived as part of the Carcinogenesis Collaborative Contract Program in 1962. In its effort to identify and evaluate chemical and physical carcinogens to which the human population may be exposed, the Bioassay Operations Segment continues to screen selected environmental agents either singly or in combination, as well as attempting to develop improved bioassay models. The activities pursuant to these objectives are: (1) identification and selection of chemical and physical agents for bioassay; (2) development of logistic capabilities to supply test agents of known purity and in sufficient quantities; (3) development of logistic capabilities to supply quality animals of known genetic background; (4) development of improved bioassay animal models and short-term tests for use as either presumptive prescreens or definitive screens; (5) development of data handling, storage and retrieval capabilities; (6) selection of suitable testing facilities; (7) implementation of approved bioassay protocols; (8) performance evaluation of testing facilities; (9) analysis, evaluation

and reporting of test results; and (10) decisions as to further action based on the evaluated data. Each of these stages requires constant reexamination for increasing its efficiency and improving its scientific quality.

Prior to the initiation of the long-term experiments, it is usually necessary to determine the acute toxicity and maximum tolerated dose levels of the test agent to properly select the chronic treatment levels. Careful attention are given to experimental design and methods used to assure adequacy of sample size, controls, dose levels, animal care, pathology, and hazard control. Once the chronic studies are initiated, data on experimental design, clinical and survival observations, and pathology results are provided to the Carcinogenesis Bioassay Data System (CBDS). Data analysis of the results can be conducted at any time during the study, but usually at their conclusion. Finally, the results are evaluated by a panel to consider the significance of the results and to recommend further action. Reports of the testing results are published in the open literature and in progress reports submitted by individual contractors.

Ongoing Program

During the year the Bioassay Operations Segment was responsible for 22 contracts plus Projects 8 and 12 at the Frederick Cancer Research Center. A number of contract actions during this past year resulted in the merger of two concurrent bioassay contracts at each of three testing facilities (Litton Bionetics, Hazleton Laboratories and Mason Research Institute); two new contract awards (Temple University to investigate U.V. photocarcinogenesis and Tracor Jitco, Inc. as a Prime Contract); two contracts transferred to other areas (New York University Medical Center to the new Smoking and Health Program and Wolf Research and Development to the Information and Resources Segment); one contract transferred into the Segment (Stanford Research Institute from the Biological Models Segment); and three contracts awarded only terminal year funds to phase out their ongoing efforts (Medical College of Georgia, New York University Medical Center, and Papanicolaou Cancer Research Institute). As a result of these actions, at the end of FY-74 the Bioassay Operations Segment was responsible for 16 contracts plus the large-scale bioassay program (Project 8) and animal farm (Task 12) at the NCI Frederick Cancer Research Center. At the year's end, the Prime Contractor was responsible for 3 of the 9 subcontracts scheduled to eventually come under its management.

At mid-year approximately 450 industrial and environmental chemicals were being screened for carcinogenicity. Of the total, about 92 were pharmaceuticals, 91 industrial chemicals, 71 pesticides and agricultural agents, 44 metallo compounds, 11 natural plant materials, 9 food additives, and 132 miscellaneous chemicals. During the year approximately 110,000 animals were used in the Bioassay Program. These include about 50,000 rats of the Osborne-Mendel, Fischer/344 and Sprague-Dawley strains; 55,000 mice of the Swiss, C57BL/6, and hybrid B6C3F1 strains; and 5,000 hamsters and other species.

Of the contracts supported during the year, eight are primarily standardized bioassay efforts in which industrial and environmental agents are tested singly and four are cocarcinogenesis efforts. Another contract effort is investigating the carcinogenicity of plants obtained from geographical regions

where they may be associated with esophageal cancer. Three other contract efforts are concerned with the development of improved bioassay models. An effort recently initiated will investigate the effect of changes in the "effective thickness" of stratospheric ozone on U.V. photocarcinogenesis. In support of the Bioassay Program, two resource contracts were funded as well as a Prime Bioassay Contract to manage the Segment's routine bioassay projects.

Bioassay Prime Contract

During the past year considerable effort was expended on the selection of a Prime Contractor for the Bioassay Operations Segment. The RFP was issued on June 11, 1973 and a 90-day proposal preparation period was given. To clarify questions of potential bidders, a pre-proposal conference was held August 9, 1973. Five proposals were received, all from commercial organizations. An ad hoc evaluation group, consisting of one-half non-NIH consultants, evaluated the proposals. Tracor Jitco, Incorporated was selected as the successful bidder and the contract was effective on March 1, 1974. During the first contract year, the Prime Contractor will assume responsibility for nine current bioassay contracts. In addition, the Prime Contractor will coordinate activities of the other Segment contractors as well as most of the Program's resources. It is expected that this arrangement will relieve the Carcinogenesis Staff from routine administrative burdens and thus allow for more scientific involvement in the program.

New Program Activities

Major undertakings of the Bioassay Program have been the review of testing protocols, standards for animal care, and procedures for data presentation, analysis and dissemination. Support has come mainly from the Carcinogen Bioassay and Program Resources Branch and the Information and Resources Segment. A workshop on "Standards for Successful Long-Term Rodent Studies" was held to review current methods of animal husbandry used in long-term carcinogenicity tests. The standards as developed will soon be published by the Institute of Laboratory Animal Resources (NAS/NRC).

The testing protocols used in long-term carcinogenesis bioassay were reviewed by a working group which met at the NCI late last fall. The Segment Director is also Chairman of an "Interagency Sub-Task Group on Carcinogen Testing" which will review the NCI revised protocol in relation to those of other agencies. Hopefully, a degree of uniformity can be achieved that meets the general needs of the government and industry.

Bioassay Reporting and Data Analysis

One of the most important aspects of the Bioassay Program is the recording, analysis, and reporting of data resulting from contract laboratories. Data from studies initiated in the past 1 1/2 years are entered into the computerized Carcinogenesis Bioassay Data System (CBDS). Data from earlier studies are formalized in progress or other written reports. The CBDS is used to collect, monitor, and store experimental data and results from bioassay operations. It is designed for the complete or selective recall

of data. The system is now in full operation so that new bioassay experiments are entered as they are initiated. Data from about 20 contractors are now on file with several other contractors preparing data for entry into the system. During this year the staff expanded the system to permit the analysis of carcinogenesis data using those methods best suited for their interpretation. In addition, the CBDS contains chemical and bioassay contract information sub-systems which list data on material presently under test or projected for study, date initiated, etc. The routine data entry, SNOP-coding, etc., are provided under contract (Contractor: Wolf Research and Development Corporation).

All contractors are required to provide an annual detailed progress report four months prior to termination date. Brief but informative interim progress reports are required every four months. Because the Bioassay Program and many contractors do not possess inhouse capability for properly analyzing test results, statisticians from other areas of the NCI have been asked to assist. Staff members in the Field Studies and Statistics Program (DCCP) have provided the bulk of assistance in this area.

Although contractors are encouraged to submit their testing results to scientific journals having wide dissemination, it is important that the results be properly tabulated and analyzed prior to their release to the public or other federal agencies. For this reason, all bioassay data must be reviewed and approved prior to their release. The Scientific Coordinator for Environmental Carcinogenesis assists in discussing bioassay results with other federal agencies. Because of their nature, dissemination of complete Bioassay data is difficult and therefore, are rarely made available in their entirety to the scientific community. To evaluate possible methods of more effectively presenting such data, a "Data Dissemination Conference" was held in January 1974. As a result of this conference, it is anticipated that practical methods can be developed.

Personnel Requirements

To effectively manage and monitor the Bioassay Program requires expertise in and coordination of various disciplines such as animal care, chemistry, toxicology, biometry and data analysis, pathology, information handling, and fiscal operations. While the Carcinogen Bioassay and Program Resources Branch and cooperating groups in Carcinogenesis possess several of these disciplines, the need for more staff is urgent in view of the projected expansion of bioassay and carcinogenesis activities. Last year it was planned to add several additional personnel to the staff to meet current needs. Unfortunately, a reduction in staffing level for the Carcinogenesis Program was necessary. To partially offset this setback, certain functions are now being accomplished by the contract mechanism. These include pathology and information contracts as well as a Bioassay Prime Contract. It is apparent that more personnel are needed to do an effective job in managing and coordinating this major research program of national importance. While the use of support contracts may help considerably, additional staff is still desperately needed to adequately perform the tasks required and monitor the overall program.

Bioassay Contract Highlights

Some major findings in the Bioassay Program during the past year include the following:

At the Medical College of Georgia a variety of known chemical carcinogens are being used to investigate the suitability of seven different mouse strains for routine bioassay models. Based on seven-week subchronic toxicity studies, no single strain was, across-the-board, the most or least tolerant to all of the test agents. DBA/2N mice would appear not to be suitable for long-term bioassay as they develop a high incidence of "spontaneous calcific pericarditis."

At Gulf South Research Institute, 20 pesticides are being tested for chronic toxicity and carcinogenicity. Based on necropsy findings, several appear to be carcinogenic. These findings are now being verified by histopathology and statistical analysis.

At Hazleton Laboratories, 16 agricultural chemicals are being investigated for chronic toxicity and carcinogenicity. Two of them, ethylene dibromide and dibromochloropropane (DBCP), both halogenated aliphatic fumigants, were found to be highly carcinogenic in both mice and rats. Tumors were predominantly squamous cell carcinomas and typically located in the stomach with metastases to adjacent viscera. DBCP also induced mammary adenocarcinoma in rats.

At Howard University 51 plants, representing 49 genera and 54 species, are being screened for carcinogenicity. These plants come mainly from the Island of Curacao in the Caribbean where the local population has an unusually high incidence of esophageal carcinoma. Since many of these plants are used as folk remedies, an association is suggested between their ingestion and this relatively uncommon form of cancer. Three of the four plants tested by subcutaneous injection into rats have demonstrated carcinogenicity. Further studies are planned using conditions more closely resembling the human mode of exposure.

At the IIT Research Institute a series of chlorinated dibenzodioxins are being studied for carcinogenicity. These extremely toxic environmental contaminants are highly stable and tend to accumulate in the food chain. They have been found in certain pesticides and other environmental chemicals. Long-term bioassays of the dibenzodioxins, administered in the diet and drinking water, are underway in both mice and rats as well as skin carcinogenesis studies in mice.

At Litton Bionetics, 50 industrial and environmental chemicals are being administered to mice and rats by either gavage, intraperitoneal injection, or in the diet. Based on necropsy results and preliminary histopathology, several of the chemicals appear to be carcinogenic. Complete histopathology and statistical analysis of the data will be necessary to verify these findings.

At the New York University Medical Center, a variety of environmental alkylating agents are being investigated for carcinogenicity as well as for structure-activity relationships. Almost 75% of the compounds given by subcutaneous injection produced local sarcomas whereas less than 15% tested by skin application induced cancer. The poor correlation between the carcinogenicity of the agents when given by the different routes of exposure indicates the need for caution in designing experiments and interpretating the results. One of the agents found to be carcinogenic by s.c. injection, epichlorohydrin, is widely used in the chemical industry in the manufacture of copolymers.

Another project at the New York University Medical Center is investigating tumor-promoting agents and cocarcinogens from tobacco tar and cigarette smoke condensate. Results from skin carcinogenesis experiments showed very few of the agents tested have tumor-promoting ability. When tested as cocarcinogens with benzo(a)pyrene, however, several of the agents produced tumor responses greater than the B(a)P control. None of the cocarcinogens tested by itself produced a notable tumor response.

At the Southern Research Institute 28 chemotherapeutic agents and related chemicals are being evaluated for chronic toxicity and carcinogenicity. Each of the test agents are administered by a route comparable to the mode of human exposure. The anticancer agents, actinomycin-D and mitomycin-C, induced statistically significant numbers of peritoneal sarcomas in CRCD Sprague-Dawley rats. Melphalan and DIC also were carcinogenic in both the rats and Swiss mice.

CONTRACT NARRATIVES

BIOASSAY OPERATIONS SEGMENT

July 1, 1973 through June 30, 1974

CINCINNATI, UNIVERSITY OF (NO1-CP-33202)

Title: Study of the Carcinogenic and Co-Carcinogenic Properties of Industrial Chemicals

Contractor's Project Director: Dr. E. Bingham Mattheis

Project Officers (NCI): Dr. Henry Hennings
Dr. Norbert P. Page

Objectives: The purpose of this project is to test for carcinogenicity and co-carcinogenicity of selected industrial chemicals to which there is significant human exposure.

Major Findings: The effect of energy dose, wavelength, animal strain on the photo-carcinogenesis dose response curve, and whether the dose response curve is modulated by the topical application of certain chemicals is under investigation. A class of fluorescent compounds which are widely used as whitening agents are being examined. A number of experiments are near completion to determine the effect of different compounds, solvents, strains, wavelengths and total doses of energy on the dose response curve.

A method of generating coal particles and coating the particles with coal tar for inhalation has been developed. Animals are currently being subjected to the inhalation of coal dust coated with coal tar, with and without the subsequent inhalation of n-dodecane. The concentration of coal dust is 18 mg/m^3 respirable dust. The concentration of coal tar sampled from the chamber is 65 mg/m^3. The level of n-dodecane is 600 mg/m^3.

Moribund animals are killed and a complete autopsy performed. All tumors are saved for histopathological examination. It is anticipated that exposures will continue over at least a year of exposure and held for eighteen months to allow for the development of tumors.

The characterization of the particles in the chamber is in progress. It has been determined that 83% of the particles in coal dust-tar mixture are 1.0 micron or less in diameter.

Fractionation of the coal tar used in this inhalation experiment has been accomplished and these components are being applied topically to the skin of mice to determine their relative carcinogenic potency.

Significance to Biomedical Research and the Program of the Institute: The chemicals under investigation in this contract are found in the environment (home or occupational) of human beings. It is essential to know whether

these materials are carcinogenic or whether they enhance or inhibit the potency of environmental carcinogens, such as ultraviolet light. A most important function of this contract is to determine how combined exposure to various agents may affect their carcinogenicity. It has been demonstrated, for example, that chemicals which are not carcinogenic when tested alone may markedly enhance the carcinogenicity of other materials. This observation has most important implications for programs designed to restrict human exposure to carcinogens and co-carcinogens.

Proposed Course: The experiments underway will be completed and further experiments on the carcinogenic and co-carcinogenic potency of industrial chemicals will be started.

Date Contract Initiated: September 11, 1963

Current Annual Level: $94,828

DOW CHEMICAL COMPANY (NO1-CP-23254)

Title: Carcinogenesis Bioassay of Environmental Chemicals

Contractor's Project Director: Dr. J. L. Emerson

Project Officer (NCI): Dr. Thomas P. Cameron

Objectives: To evaluate the potential carcinogenicity and chronic toxicity of 5 selected environmental chemicals used in commerce and industry by chronic bioassay in F344/CR rats and B6C3F1 mice.

Major Findings: The acute oral median lethal dose and subacute 6 weeks dietary studies have been completed in rats and mice for 2-amino-5-nitrothiazole, 1,3-dichloro-5,5-dimethylhydantoin, 3-nitropropionic acid, proflavine dihydrochloride, N,N^{1}-dicyclohexylthiourea and resorcine blue. In addition, acute and subacute studies on scopolamine hydrobromide have been completed in mice. Resorcine blue and scopolamine hydrobromide have been dropped from further testing and replaced with N,N^{1}-dicyclohexylthiourea.

From the results of the subacute dietary studies, the maximum tolerated dose (MTD) was determined for each chemical in each species. The MTD and 1/2 MTD are being used in the current chronic rat studies of 110 weeks and mouse studies of 90 weeks duration. Each dose level consists of 50 males and 50 females of the two rodent species. Due to a lack of stability of 3-nitropropionic acid in the diet, this chemical is being administered 5 days per week by gavage. All other chemicals are being administered in the diet, which is prepared weekly or biweekly. N-2-fluorenylacetamide is being used as a positive control. Overt toxicity characterized by an acute hemorrhagic gastritis, gastric dilatation and death occurred in rats and mice that received 1,3-dichloro-5,5-dimethylhydantoin by gavage during the subacute tests. Therefore, the route of administration was changed to dietary. All chemicals are in the chronic stage of testing, which began in March and June of 1973.

To date, survival has been good in rats and female mice. Approximately 2% mortality has occurred in male mice due to fighting. Hepatic or lymphoid tumors have been observed in all animals dying from the positive control. Dose related weight suppression has been observed with some chemicals.

Significance to Biomedical Research and the Program of the Institute: A primary effort in the prevention of cancer consists of determining potential carcinogenic hazards in the environment so that human use and exposure may be controlled. The aim of this program is to gather such data for a number of widely used materials.

Proposed Course: All chemicals are currently in the stage of chronic testing which will continue into 1975. All animals will be examined at the time of death or at the termination of the study for evidence of neoplasia and chronic toxicity. This is the second year of the contract which is projected to continue through Fiscal Year 1975.

Date Contract Initiated: June 12, 1972

Current Annual Level: $99,004

GEORGIA, MEDICAL COLLEGE OF (NO1-CP-23256)

Title: Carcinogenesis Bioassay of Environmental Chemicals

Contractor's Project Director: Dr. Y. Akamatsu

Project Officers (NCI): Dr. Thomas P. Cameron
Dr. James M. Sontag

Objectives: To determine the suitability of several strains of mice for future use in carcinogenicity bioassay tests of chemicals.

Major Findings: The subacute toxicity tests of 22 known chemical carcinogens to determine the maximum tolerated dose (MTD) have been completed on seven strains of mice by continuous gavage feeding for seven weeks. There was no "absolute sensitive" strain to all chemicals being tested, but the general sensitivity was as follows in decreasing order: DBA/2, C57BL/6, C3Hf/He, BALB/c, B6C3F1, CBAf/LWN and BTOs.

Pathological observations on toxic deaths could be classified as (a) acute toxic deaths and (b) subacute toxic deaths. The acute toxic deaths were those that occurred within the first three weeks of treatment and the pathological findings were characterized by extensive autolytic changes of visceras.

The subacute toxic deaths were those observed after the three weeks of treatment, and revealed qualitative differences of pathological findings between chemicals; therefore, the 22 chemicals could be classified as three groups, (a) hepatotoxic, (b) gastrointestine-toxic (G.I. toxic) and (c) hematopoietic-toxic. In the hepatotoxic group, pathological changes were a

complex of coagulation necrosis of parenchymal hepatic cells (in eight chemicals), proliferation of bile-ductules (in six chemicals) and pericholangitis (in methylnitrosonitroguanidine). In the G. I. toxic group (three chemicals), chemical gastroenteritis was evident, and it led to shock status and death, probably due to metabolic acidosis. In the hematopoietic-toxic group, hemosiderosis and/or pigmentation of reticular tissue (in nine chemicals) and depletion of myelogenous as well as lymploid tissue (in 15 chemicals) were observed.

Animals surviving after seven weeks of treatment showed more regenerative changes than degenerative ones. In the liver, there was proliferation of the bile ductules and increased number of diploid, small hepatic parenchymal cells, especially in safrole, nitrosodibutylamine, and nitrosodiethylamine. In the G. I. tract toxic group, acanthomatous proliferation of the forestomach and intestinal metaplasia in glandular stomach, (four chemicals and three chemicals, respectively) were observed. In the hematopoietic-toxic group, either or both myeolgenous tissue and/or lymploid tissue proliferation occurred from 15 chemicals. Occasionally, acute cystitis, regeneration of renal tubular epithelium, and depletion of colloid from the thyroid glands were also observed.

Qualitative differences among the strain of mice were not as evident as those of quantitative differences in this phase of the study.

Significance to Biomedical Research and the Program of the Institute: Currently, the program of Bioassay Operations Segment uses B6C3F1 hybrid mice as one of its standard testing animals. This project is designed to investigate if a more suitable strain of mouse is available.

Proposed Course: This contract will be phased out because of the unavailability of suitable animal facilities at the Medical College of Georgia for the containment of the hazardous chemicals being tested.

Date Contract Initiated: June 29, 1972

Current Annual Level: $85,644

GULF SOUTH RESEARCH INSTITUTE (NO1-CP-43267)

Title: Carcinogenesis Bioassay of Pesticides and Other Environmental Chemicals

Contractor's Project Director: Dr. Harry P. Burchfield

Project Officers (NCI): Dr. Herman Kraybill
Dr. Thomas P. Cameron

Objectives: The objective of this project is to test a number of commonly used pesticides for chronic toxicity and carcinogenicity in mice and rats by administration in the diet.

Major Findings: Range-finding studies on the toxicity of 20 pesticides and three positive controls have been completed using Osborne-Mendel random bred rats and C57BL6 x C3H/He hybrid mice. All of the compounds are being tested for carcinogenicity at the highest tolerated dose and at one-half this value using a total of 5000 rats and 5000 mice. Pesticides are withheld from the diet at the end of 80 weeks, and the mice are sacrificed at 90 weeks and the rats at 110 weeks for gross and histopathology. All new batches of pesticides received for toxicology studies have been analyzed chemically to establish identity and the presence of impurities. Methods have been developed and applied for the analysis of endogenous pesticides and PCBs in feed as well as the amounts of pesticides added to feed.

Sufficient photodieldrin for the entire program has been synthesized. Stabilities of all compounds in the mixed feed have been established, and all feeds have been analyzed routinely for accuracy of dosage, mixing, and stability. The basic feeds from several of the contracting laboratories, including GSRI, have been analyzed routinely for endogenous pesticides and PCBs.

Sacrifice of mice and rats, gross necropsy, and histopathology are being conducted as per schedule for the first group of 23 compounds placed on test. A comparison between the incidence of tumors and tumor-like lesions observed at necropsy in test and negative control animals appears to indicate that some compounds showed carcinogenic activity. These findings must be confirmed by histopathological examination.

Significance to Biomedical Research and the Program of the Institute: Many of the pesticides under study have widespread usage resulting in extensive human exposure to them. The chemicals selected for testing have either been tested inadequately for carcinogenicity in the past or the results of such tests have been equivocal. These extensive experiments are designed to yield definitive data and help resolve these issues.

Proposed Course: All chemicals have been analyzed and are presently being evaluated for carcinogenicity to rats and mice. Sacrifice of the animals for histopathologic examination will continue during 1974.

Date Contract Initiated: June 15, 1970

Current Annual Level: $583,800

HAZLETON LABORATORIES (NOI-CP-43245) FORMERLY: (NOI-CP-33225 and NOI-CP-23278)

Title: Carcinogenesis Bioassay of Pesticides and Other Chemicals in the Environment

Contractor's Project Directors: Dr. Marcelina B. Powers
Dr. Richard W. Voelker

Project Officer (NCI): Dr. Norbert P. Page

Objectives: To determine the carcinogenicity of pesticides and environmental chemicals in Osborne-Mendel rats and B6C3F1 mice by administration in the diet and by oral intubation at the 1/2 MTD and MTD levels.

Major Findings: Fifteen of the 16 pesticides under study have completed the 90-week mouse and 110-week rat carcinogenesis trials. Histopathology has been completed in both species for Trifluralin, Chlorobenzilate, Dioxathion, and the positive control material, AAF. Data on these chemicals will be statistically analyzed. Results suggest that these three pesticides are not carcinogenic in rats and mice; the number of tumor-bearing animals and the types of tumors produced were comparable with those noted in the controls. AAF produced the expected carcinogenic effects in rats and mice. Non-neoplastic lesions were generally those associated with age; however, compound-related testicular atrophy was noted in 75% of Chlorobenzilate-treated rats. This lesion was not seen in the mice.

Eighteen industrial chemicals, mostly volatile, halogenated solvents, are being evaluated for carcinogenicity by chronic oral intubation to Osborne-Mendel rats and B6C3F1 mice. The 90-week carcinogenesis trial has been completed in mice for three chemicals. The 78-week treatment phase has been completed in rats for 15 chemicals and in mice for eight chemicals.

Two halogenated aliphatic fumigants, Dibromochloropropane (DBCP) and Ethylene Dibromide, were found to be highly active carcinogens in both rodent species. Tumor induction in rats was observed as early as 10 weeks after initiation of treatment with Ethylene Dibromide and 14 weeks with DBCP. Both chemicals produced a high incidence of squamous cell carcinomas in rats and mice (54 to 100% of all histologically diagnosed tumors). These tumors were predominantly located in the stomach with metastases to adjacent visceral organs. DBCP also induced mammary adenocarcinomas in rats.

Significance to Biomedical Research and the Program of the Institute: A primary effort in the prevention of cancer consists of the identification of potentially carcinogenic hazards in the environment so that the human exposure to them may be minimized. The chemicals under study have widespread usage in industry and in some cases are components of household products resulting in extensive human exposure. These studies are an attempt to evaluate their carcinogenic potential. If found to be carcinogenic, appropriate action can be taken to control their use. Information obtained will also be valuable in establishing precautionary measures for safe handling and usage of these chemicals.

Proposed Course: Histopathology will be completed on the pesticides evaluated on this program. The feeding trials on the remaining positive control material, Aminotriazole, will continue. Chronic oral intubation will continue in rats and mice for the remaining halogenated industrial chemicals, responses will be carefully evaluated, and each study will be terminated as scheduled. Statistical analyses will be performed on compiled data on the combined program.

Further work is planned on the two carcinogenic fumigants, Ethylene Dibromide and DBCP, utilizing the inhalation route. Acute and subchronic inhalation

exposures in Fischer rats and B6C3F1 mice are scheduled preliminary to chronic (lifetime) exposures.

Date Contract Initiated: February 1, 1974 (December 1, 1970, NO1-CP-33225; May 1, 1971, NO1-CP-23278)

Current Annual Level: $990,600

HOWARD UNIVERSITY (NO1-CP-33266)

Title: Chemical and Biological Investigation of Potential Carcinogens from Plants

Contractor's Project Director: Dr. Govind J. Kapadia

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: (1) To prepare purified fractions from certain herbs that are used habitually in Curacao and South Carolina and isolate and characterize crystalline compounds from these fractions, (2) to test each fraction and compound for carcinogenicity in experimental animals and identify substances responsible for the carcinogenic effects, (3) to extend such chemical and bioassay studies to plant species related to the above as well as to other plants that are suspected of possessing carcinogenic activity, and (4) to elucidate the chemical structure of any carcinogenic substance that may be isolated.

Major Findings: This project is a continuation of an earlier investigation which was developed in an attempt to correlate the high incidence of esophageal carcinoma in natives of certain places with their habit of using herbaceous folk remedies. Thus far, 33 plant samples (belonging to 28 genera and 31 species, procured from Curacao, South Carolina, and other places) have been extracted with water, the aqueous extracts lyophylized and submitted for bioassay. The aqueous extracts of tannin-bearing plants were fractionated to yield tannin and tannin-free fractions which were also provided for bioassay. Tannins of Krameria ixina, K. triandra, Acacia villosa, Myrica cerifera, and Diospyros virginiana were chemically examined. Acid hydrolysis of tannin resulted in identification of phloroglucinol and catechol from the two Krameria species, resorcinal and catechol from A. villosa, phloroglucinol and gallic acid from M. cerifera and gallic acid and glucose from hydrolyzable tannin, and phloroglucinol and gallic acid from condensed tannin of D. virginiana. Crystalline or other products isolated from fractions of A. villosa, Sorghum vulgare, M. cerifera, Melochia tomentosa, Sassafras albidum, and Tribulus cistoides were partially or completely characterized.

Bioassay of several extracts and their fractions were carried out by subcutaneous injections in suitable doses to NIH black rats once a week. A total of eleven doses of eight extracts and fractions from four plants have been used. The observation period ran up to 75 weeks for these groups during which development of tumor was detected in treated rats. The extracts and fractions that produced tumor and the ratio of the number of rats developing tumor to the

total number used are as follows: K. ixina aqueous extract (54/90), tannin-free fraction (3/60), and tannin (28/30); K. triandra aqueous extract (30/30), and tannin (28/30); Sorghum vulgare (bird resistant variety), aqueous extract (0/30); A. villosa aqueous extract (30/30), and tannin (29/30). Among these plants, A. villosa appeared to be the most potent carcinogen. Histologically, the induced tumors showed characteristics of malignant fibrous histiocytomas that manifested low grade malignancy but usually had a multi-nodular growth pattern.

Significance to Biomedical Research and the Program of the Institute: This project should serve as the ultimate check to ascertain the carcinogenicity of materials implicated by epidemiological studies on the island of Curacao. Information gained from this study could conceivably lead to definitive studies that should be conducted in other high incidence areas to identify the carcinogens involved.

Proposed Course: During the coming period, plant extracts will continue to be prepared and tested for carcinogenicity. To increase the rate of bioassay, instead of screening each fraction, only the total extract will initially be tested. If this shows carcinogenic activity, then its fractions will be studied. An attempt will be made to isolate and identify the active component(s) from carcinogenic fractions.

Date Contract Initiated: June 25, 1971

Current Annual Level: $133,528

IIT RESEARCH INSTITUTE (NO1-CP-12338)

Title: Carcinogenesis Bioassay of Chlorinated Dibenzodioxins and Related Chemicals

Contractor's Project Director: Dr. Maurice E. King

Project Officers (NCI): Dr. Herman F. Kraybill
Dr. Richard R. Bates

Objectives: To determine the carcinogenicity and co-carcinogenicity of chlorinated dibenzodioxins and related compounds by application to the skin of mice and by oral administration to mice and rats.

Major Findings: Tests to determine the potential carcinogenicity of the non-toxic dioxins by skin application have been completed and microscopic examinations of the mice are in progress. Bioassay of these compounds as complete carcinogens involved application of the test compound, dissolved in acetone, to the backs of mice three times a week for 78 weeks. A similar procedure was used for assay of the dioxins as promoting agents except that mice were given a single application of 50µg of DMBA prior to application of the test compounds.

Mice from all treatment groups have exhibited skin as well as internal lesions, however the incidence and spectrum of lesions seen so far in the dioxin-treated mice is considerably less than those found for the croton oil positive control group and the dioxane promotion group. Additional groups are now on test to recheck some of the earlier findings and to determine any possible effects of inhalation exposure on the results.

Feeding studies with unsubstituted and 2,7,-dichlorodibenzo-p-dioxin are nearing completion. However, numerous restarts have been necessary with octachlorodioxin. The 1.0% and 0.5% dietary levels used with the other compounds are too high for octachlorodioxin, as evidenced by excessive weight loss and mortality. Male mice have tolerated 0.1% and 0.05% dietary octachlorodioxin for over five months, and female mice have received 0.5% and 0.25% for over a year without an appreciable effect on weight or excessive mortality. Weight loss and deaths began to occur after 3 months among female rats at 0.1 and 0.05% octachlorodioxin. Some deaths have occurred with male rats at 0.25% and 0.1% octachlorodioxin. However, after 5 months they exhibit a higher tolerance than the females. Generally, mortality and the number of survivors bearing palpable tumors indicate that females of both species are more sensitive to the dioxins by oral administration.

Preliminary tests are now in progress to determine the dose levels to be used for skin and oral studies with the toxic tetrachloro-, hexachloro-, and trichlorodibenzo-p-dioxins. After evaluation of these results, chronic studies analogous to those with the non-toxic dioxins will be initiated.

Significance to Biomedical Research and the Program of the Institute: Chlorinated dibenzodioxins have been found as extraordinarily toxic contaminants in certain pesticides and other environmental chemicals. In addition to their extreme toxicity, they are highly stable under environmental conditions and tend to accumulate in the food chain. They have been tested for acute toxicity and teratogenicity but have not been examined for carcinogenicity. This program is designed to accomplish the latter.

Proposed Course: As sufficiently pure samples of the other chlorinated dibenzodioxins selected for study become available, they will be entered into chronic tests. Preliminary investigations on the feasibility of testing the related chlorinated dibenzofurans will be carried out. Proposed long-term experiments should require three more years for completion.

Date Contract Initiated: June 25, 1971

Current Annual Level: $346,356

LITTON BIONETICS, INC. (NO1-CP-43349) FORMERLY: (NO1-CP-23252 and NO1-CP-12146)

Title: Carcinogenesis Bioassay of Pesticides and Other Environmental Chemicals

Contractor's Project Director: Dr. F. M. Garner

Project Officer (NCI): Dr. Norbert P. Page

Objectives: To study the chronic toxicity and possible carcinogenicity in rats and mice of 40 chemicals presently utilized in industry or present in man's environment.

Major Findings: Chronic studies are now underway with 36 chemicals. While most are administered in the feed, some are given by gastric intubation and intraperitoneal injection. One rat study has been completed and tissues are being processed for histologic review. Gross necropsies revealed a high incidence of malignant hepatic neoplasms in the treated animals. Such lesions are rare in the controls. The levels to be used for chronic experiments will be established in the remaining compounds with all compounds to be on test soon.

Significance to Biomedical Research and the Program of the Institute: This project, along with others in the Bioassay Operations Segment, is intended to identify potentially carcinogenic substances present in the environment. By identifying these carcinogenic agents it may be possible to institute measures to minimize man's exposure by prohibiting or restricting the use of these agents or modifying the method by which they are processed and handled.

Proposed Course: Chemicals are fed to rats and mice over their lifespan at a maximum-tolerated-dose and at 1/2 that level. The animals will be sacrificed after 24 months in the study, and all animals will be examined for evidence of neoplasia and chronic toxicity. As these are long-term studies, and some of the experiments are only now beginning, the project is expected to continue through 1976.

Date Contract Initiated: February 1, 1974 (May 13, 1971, NO1-CP-12146 June 29, 1972, NO1-CP-23252)

Current Annual Level: $299,570

NCI, FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc. (NO1-CP-23294)

Project #8

Title: Large Scale Bioassay

Contractor's Project Manager: Dr. Borge Ulland

Project Monitor (NCI): Dr. Norbert Page

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis.

MASON RESEARCH INSTITUTE (NO1-CP-43297) FORMERLY: (NIH-NCI-71-2144 and NIH-NCI-72-3255).

Title: Carcinogenesis Bioassay of Environmental Chemicals

Contractor's Project Director: Dr. Emil R. Smith

Project Officers (NCI): Dr. Thomas P. Cameron
Dr. Elizabeth K. Weisburger

Objectives: To study the chronic toxicity and possible carcinogenicity of environmental chemicals.

Major Findings: A total of 50 compounds or mixtures of compounds are being fed in the diets of the Fischer 344 rats and either C57Bl/6 mice or B6C3Fl mice. The compounds are being fed singly or, in some cases, as combinations of two compounds in order to determine whether or not they are potentially carcinogenic as they occur in the environment.

These studies have been in progress for many months but are not yet sufficiently complete that conclusions can be drawn on the basis of statistical analysis of the data. However, tentative conclusions can be made concerning tumor production in animals fed some compounds or mixtures. Dulcin alone and in combination with arginine glutamate appeared to produce carcinomas of the urinary bladder of rats but not mice; acetamide appeared to produce hepatomas in both rats and mice and lymphomas in mice; and a combination of n-butyl urea and sodium nitrite produced an array of tumors in rats, including leukemias and carcinomas of the ear duct and forestomach. In mice, this mixture appeared to produce lymphomas. Finally, it appears that 2-methyl-1-nitroanthraquinone may have produced hemangioendotheliomas, hemangiosarcomas and angiosarcomas, and possibly sarcomas in mice.

Significance to Biomedical Research and the Program of the Institute: A major function of the Carcinogenesis Program is to evaluate the potential carcinogenic risk represented by chronic exposure of man to environmental chemicals. This contract, by assessing the carcinogenicity of some of these substances in animals, will identify compounds which are potentially hazardous to man.

Proposed Course: Studies on 12 compounds or mixtures will be completed during 1974; studies on an additional 20 compounds should be completed in 1975; and studies on the remaining compounds being tested should be completed in 1976.

Date Contract Initiated: January 1, 1974 (May 1, 1971, NIH-NCI-71-2144; June 29, 1972, NIH-NCI-72-3255)

Current Annual Level: $631,315

MIDWEST RESEARCH INSTITUTE (NO1-CP-23270)

Title: Analytical Chemistry Resource

Contractor's Project Director: Dr. Evelyn Murrill

Project Officers (NCI): Dr. Marcia Litwack
Dr. Larry Keefer

Objectives: The primary objective of this program is to provide state-of-the-art analyses of chemicals, chemical/feed mixtures, and feeds for the Carcinogenesis Program. The secondary objective is to purchase, store, and distribute bulk chemicals for use in the Carcinogenesis Program.

Major Findings: During the first 20 months of the contract, 85 chemical requests have been received for procurement, distribution, storage, and analysis; 40 requests for analysis only; 9 requests for purchase and distribution only; and 59 requests for procurement in bulk amounts, distribution, storage, and analysis of previously requested compounds. Suppliers have been located for 79 chemicals, and the chemicals have been purchased; six requests have been delayed or cancelled because of synthetic difficulties or cost. Analysis has been completed on 103 chemical samples and 30 chemical/feed mixtures. Currently, 22 other samples are being analyzed.

Analysis of chemical samples includes complete physical-chemical analysis of the nominal ingredient as well as assay and characterization of impurities. Chemical/feed mixture analysis includes the determination of chemical stability in dosed feed, the kinetics of degradation, and the monitoring of blending homogeneity.

Significance of Biomedical Research and the Program of the Institute: The literature of carcinogenesis bioassay studies contains many examples of experiments which cannot be meaningfully interpreted because the test substances had not been adequately characterized chemically. When such an experiment results in low tumor incidence, for example, the question will always remain as to whether the tumors observed might not have arisen from small concentrations of highly potent impurities, rather than from the allegedly weak potent nominal ingredient. Other questions of a chemical nature which are central to the interpretation of bioassay data include such problems as decomposition of the chemical in feed or intubation mixtures and levels of mycotoxins or pesticides in food and bedding. For these reasons it is essential that the Carcinogenesis Program have available to it a facility capable of characterizing those materials entered into the testing program, as well as determining the effectiveness of administration of these substances to the test animals.

Proposed Course: To continue the purchase, storage, and distribution of compounds used in the bioassay program, and the analysis of bulk chemicals, chemical/feed mixtures, and feed.

Date Contract Initiated: June 28, 1972

Current Annual Level: $140,540

NEBRASKA, UNIVERSITY OF (Eppley Institute for Research on Cancer)(NO1-CP-33278)

Title: A Resource for Carcinogenesis Bioassays and Related Research

Contractor's Project Director: Dr. Philippe Shubik

Project Officer (NCI): Dr. Gio B. Gori

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis.

NEW YORK UNIVERSITY (NO1-CP-33241)

Title: Studies on Carcinogenesis Principles of Processed Tobacco and Tobacco Smoke

Contractor's Project Director: Dr. Benjamin L. Van Duuren

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: To determine the nature of the carcinogenic, co-carcinogenic and tumor-promoting agents that are known or suspected components of various cigarette smoke condensates. These also include possible other deleterious factors.

Major Findings: In past years much emphasis has been placed on fractionation of tobacco tars and tests for tumor-promoting agents of these fractions or of pure compounds which are known constituents of cigarette smoke condensate (C.S.C.). Very few of these materials showed significant tumor-promoting activity in initiation-promotion experiments; therefore, a different type of mouse skin experiment was used. In these experiments the carcinogen and agent tested as cocarcinogen (as opposed to tumor-promoting agent) are applied simultaneously to mouse skin three times weekly. Benzo(a)pyrene (BP), a tobacco smoke carcinogen, was applied at a dose of 5 µg/application, three times weekly with the compound on test as co-carcinogen. The dose of BP selected was based on our earlier experience in dose-response studies with BP using female ICR/Ha mice; 5 µg/application of BP alone in acetone solution 3x weekly, resulted in a marginal tumor-response late in the experiment. A series of compounds: phenol, esculin, eugenol, quercetin, pyrene, benzo(g,h,i) perylene and benzo(e)pyrene were tested as co-carcinogens using this test system. The experiments were terminated after one year. None of the test compounds resulted in notable tumor incidences when applied by themselves. However, when applied together with BP the following compounds showed high tumor incidences (papillomas and cancers) compared to the BP control: benzo(e)pyrene, benzo(g,h,i)perylene, pyrene and catechol. Very significant in these results are: (a) some of these agents occur in tobacco tar in much higher concentration than BP, formerly considered the most important carcinogenic agent in tobacco tar. Catechol is the most abundant phenol in tobacco tar. An 85 mm non-filter cigarette yields up to 0.5 mg of catechol; (b) Phenol, formerly considered the important tumor promoter in cigarette tar, has indeed a tumor-inhibitory effect in co-carcinogenesis; (c) Since not all co-carcinogens are tumor promoters and vice versa, it was suggested that these two terms not be used interchangably and, moreover, findings point to different mechanisms of action of these two types of agents; (d) There is a high degree of structural specificity for co-carcinogenic activity. Thus, while catechol (1,2-dihydroxybenzene) is active, its close analog, resorcinol (1,3-dihydroxybenzene), is inactive. This finding is strengthened in additional tests now underway.

Significance to Biomedical Research and the Program of the Institute: Lung cancer is one of the leading causes of cancer death, and the importance of cigarette smoking in the etiology of this disease is well established. Therefore, the identification of the agents responsible for the deleterious health effects of cigarette smoke is of critical importance for the ultimate reduction of cancer risk due to cigarette smoking. The identification of these agents, an understanding of their biological effects, and the mode of action can lead to their removal from tobacco or cigarette smoke.

Proposed Course: The examination of the tumor-promoting and co-carcinogenic activity of a variety of phenolic and related compounds is underway. The co-carcinogenic and tumor-promoting activity of a series of alkanes, alkenes, terpenes, fatty acids and alcohols of tobacco leaf and/or cigarette smoke condensate is being evaluated. The relationship of aryl hydroxylase enzyme systems to the tumor-inhibiting activity of various agents already tested in co-carcinogenesis studies will be further examined. The mixed culture system will be reexamined with the goal of establishing a test system using other cell types. Previous efforts to grow untransformed mouse epidermal tissue *in vitro* for long periods of time have not been successful. As a part of the present work methods of growing cells derived from mouse skin papillomas or carcinomas will be explored. These tumor cells will be used in a mixed culture system along with various untransformed cell populations.

Date Contract Initiated: June 29, 1964

Current Annual Level: $113,149

NEW YORK UNIVERSITY (NO1-CP-43221)

Title: Alkylating Agents as Carcinogens

Contractor's Project Director: Dr. Benjamin L. Van Duuren

Project Officers (NCI): Dr. Elizabeth Weisburger
Dr. Lionel Poirier

Objectives: A wide variety of alkylating agents are being introduced into the environment. Some of these compounds are encountered in manufacturing processes where they may be occupational hazards. Others become distributed in the environment to greater or lesser extents. The purpose of this contract has been to determine the possible carcinogenic activity of some of these compounds in laboratory animals using several routes of exposure and to explore structure-activity relationships. The final aim of the work is to yield information that can lead to the reduction or elimination of human exposure to these agents.

Major Findings: During the past year tests have been completed on a series of chloro ethers and related compounds. The methods used were mouse skin application, initiation-promotion experiments, subcutaneous injection in mice and intraperitoneal injection for a small number of compounds. In addition, the relationship between chemical structure, chemical reactivity and

carcinogenic activity was examined. One of the unexpected results of these experiments was the poor correlation of the results between mouse skin application and subcutaneous injection. Of 15 compounds tested by skin application, only two resulted in the induction of local carcinomas whereas by subcutaneous injection, 11 of 16 compounds tested resulted in the induction of local sarcomas. These results suggest that caution be exercised in the interpretation of findings. The compounds that induced local sarcomas are as follows (% animals with local sarcomas are indicated in parentheses): ethylbromoacetate (18%), dimethylcarbamyl chloride (78%), 2,3-dichloro-p-dioxane (36%), epichlorohydrin (14%), glycol sulfate (73%), and diethyl-β,γ-epoxypropyl phosphonate (30%). Of these compounds only dimethylcarbamyl chloride (DMCC) induced a significant incidence of squamous carcinomas of the skin (60%). It has been suggested that DMCC be used as a pesticide or as an anthelmintic, presumably in veterinary medicine, but the extent of its use in chemical industry could not be ascertained. It can be potentially used in the manufacture of certain types of polyurethanes. It has both electrophilic and nucleophilic properties which suggests possible wide use in the chemical industry and, hence, a possible occupational carcinogen.

Epichlorohydrin is widely used in the manufacture of copolymers for various purposes and because of its volatility and wide use it should probably be tested for carcinogenicity by inhalation experiments.

Valuable structure activity relationships were uncovered in the course of this study.

Significance to Biomedical Research and the Program of the Institute: The accomplishments of this contract are expected to result in identifying the carcinogenic hazard to man of industrial and environmental chemicals; this will enable the reduction or preferably their complete elimination from the human environment including occupational exposure.

Proposed Course: To continue to examine halo-ethers and other related alkylating agents particularly those of industrial and environmental importance in relation to cancer causation in man. Compounds of these types will be selected on the basis of predictions made from structure-activity relationships.

Date Contract Initiated: October 1, 1970

Current Annual Level: $121,212

PAPANICOLAOU CANCER RESEARCH INSTITUTE (NO1-CP-23253)

Title: Bioassay of Potential Carcinogenic Compounds in Six Inbred Strains of Rats

Contractor's Project Director: Dr. Wilhelmina Dunning

Project Officers (NCI): Dr. James M. Sontag
Dr. Thomas R. Cameron

Objectives: To determine the suitability of several inbred strains of rats for possible use in bioassay of chemicals for carcinogenicity. A comparison is being made of their response to the chemical carcinogen, N-hydroxy-N-2-fluorenylacetamide.

Major Findings: Comparative chronic toxicity tests are underway with the chemical N-hydroxy-N-2-fluorenylacetamide in six inbred strains of rats. These include the Fischer line 344, A x C line 9935 irish, August line 990, Marshall line 520, S x F line 40814, and Zimmerman line 61.

Currently 778 rats have been force-fed N-hydroxy-N-2-fluorenylacetamide on a chronic schedule. The females have received 10.0 and 5.0 mg/Kg. by intubation on a 5-day a week schedule for six months. The males of each strain have received 5.0 or 2.5 mg/Kg on a similar schedule. About half of the animals are still alive.

Necropsy records are fairly complete for the dead rats that survived the six months of chronic feeding. A total of 145 neoplasms have been described, of which 76 have been confirmed and classified by microscopic examination. The observed neoplasms include 76 liver tumors; 35 neoplasms involving the wall of the stomach; 17 involving the face, orbit or ear canal; 5 spleen; 7 mammary gland; 1 ovary; 2 uterus; 3 bladder; and 2 involving the lymph nodes of the illio-colic mesentery. More than 50 per cent of the observed stomach tumors were in rats of the August line 990 while none were found in rats of the Marshall line 520.

Approximately 100 rats (16 of each strain) are serving as vehicle controls, receiving 1 cc of suspending vehicle five days a week for six months. Another group of 100 rats are under observation as untreated controls. One Marshall line 520 female from this group died with a sarcoma that arose in the mesentery of the illio-colic junction.

Significance to Biomedical Research and the Program of the Institute: As part of the Bioassay Operations Segment's program, many compounds are now being tested in various pure strains of rats. As an outcome of this study, more sensitive strains of rats than those presently used may be found. This would facilitate the bioassay testing program.

Proposed Course: Complete the chronic feeding of N-hydroxy-N-2-fluorenylacetamide and the necropsy records and pathology reports for the treated groups and the controls.

Date Contract Initiated: June 16, 1972

Current Annual Level: $70,565

SAN FRANCISCO, UNIVERSITY OF (NO1-CP-33229)

Title: Study of Carcinogenicity of Metallo-organic Compounds

Contractor's Project Director: Dr. Arthur Furst

Project Officers (NCI): Dr. John Cooper
Dr. Richard Bates

Objectives: The primary objective of this contract is the investigation of the carcinogenic hazard posed by metals in the environment. Long-term bioassay studies have been employed to test various metallic compounds by different routes of administration in rodents. Emphasis is placed upon a thorough evaluation of those metallic compounds which are industrial hazards, commercial contaminants, or environmental pollutants.

Additional objectives include study of possible *in vivo* methods of blocking the carcinogenic effects of certain metals by the use of other non-carcinogenic metals or cholating agents. Also, some metabolic studies are planned to investigate the excretion of carcinogenic metals and their effects upon normal trace metal concentrations in various tissues. It is hoped that such information may shed light upon the mechanisms by which certain metals cause cancer.

Major Findings: Metal powders and selected compounds have been evaluated intramuscularly and by gavage in Fischer-344 rats. A screening procedure (intramuscularly) to determine which metals are active as carcinogens has been developed. Those which show a positive reaction are then evaluated by routes more likely to parallel modes of human exposure (gavage and intratracheal instillation). To date, results of intramuscular studies show that metal powders and/or selected compounds of copper, iron, gold, silver, lead, aluminum, zinc, and tin are *not* carcinogenic. By contrast, compounds of cadmium and nickel are potent carcinogens, inducing highly malignant neoplasms (fibrosarcomas and rhabdomyosarcomas) at the injection site. Certain compounds of manganese (dioxide, acetylacetonate) are also very active carcinogens. Chromium, vanadium, and molybdenum also show carcinogenic activity when given intramuscularly. Certain selenium compounds, while failing to induce tumors at the injection site, do induce a low but significant incidence of hepatomas.

Titanium compounds are most interesting. When administered intramuscularly, some compounds are capable of inducing both fibrosarcomas at the injection site as well as lymphocytic leukemia. This leukemia is similar to that which appears spontaneously in senile Fischer-344 rats but is seen earlier and much more frequently in titanium-treated rats. Administration of titanium dioxide by gavage has resulted in the appearance of several unusual neoplasms associated with the gastrointestinal tract. These include several hepatomas, a bile duct carcinoma, fibrosarcoma, adenocarcinoma of the pancreas, and an hemangioendothelioma. Extensive additional studies are planned to evaluate other titanium compounds by gavage.

Significance to Biomedical Research and the Program of the Institute: Heavy metals and their compounds are ubiquitous in the environment, being used widely in industry and commercial (consumer) products of many types. The presence of large quantities of metallic compounds as contaminants in the atmosphere is well documented. As technology has expanded, so have the uses for metals. For example, metals or metallic compounds are used as binders in the pharmaceutical industry, as preservatives in carbonated beverages, as aviation fuel additives, as components of medical implantation devices, as catalysts in the manufacture of plastics, and in countless other ways, most of which involve extensive exposure of large populations to metals. Consequently, it is extremely important to determine precisely which metals present carcinogenic hazards in the environment and to investigate the nature of such carcinogenic effects. A thorough investigation of the mechanisms by which some metals induce cancer could provide valuable information on the essential nature of the cancer problem.

Proposed Course: The contractor will serve as a resource to the Carcinogenesis Program on the industrial uses of metals and their carcinogenicity. Long-term bioassay studies will continue on those metals which pose potential health hazards to large segments of the population. Emphasis will be placed upon compounds of titanium and manganese, together with selected other metals shown to be carcinogenic by the intramuscular route. Long-term studies will be carried out by gavage and by intratracheal instillation. Limited biochemical studies will be undertaken to investigate the metabolism and excretion of carcinogenic metals, and the effects of such metals on trace metal metabolism in selected tissues.

Date Contract Initiated: June 3, 1964

Current Annual Level: $99,744

SOUTHERN RESEARCH INSTITUTE (NO1-CP-33214)

Title: Carcinogenicity Studies of Chemotherapeutic Agents and Related Chemicals

Contractor's Project Director: Dr. D. P. Griswold

Project Officers (NCI): Dr. Norbert P. Page
Dr. Elizabeth K. Weisburger

Objectives: To evaluate the carcinogenic activity of various chemotherapeutic and related chemicals to which man is currently being exposed on a long-term basis.

Major Findings: A series of 28 chemotherapeutic agents or related chemicals are undergoing bioassay for carcinogenic activity in rats and mice. The subacute studies have been completed and 22 of these agents are currently in the chronic phase of evaluation. The animals exposed to the remaining six compounds have recently completed the chronic study and are awaiting histopathology.

Compounds under evaluation include anticancer agents such as Myleran and acronycine, all of the major oral hypoglycemic agents, and various miscellaneous chemotherapeutic drugs or related chemicals such as emetine (an amebacide), Dibenzyline (an anti-hypertensive), phenazopyridine (a urinary analgesic), 4-4'-sulfonyldianiline (an antimicrobial), and 4,4'-thiodianiline (an intermediate in the production of 4,4'-sulfonyldianiline).

Significance to Biomedical Research and the Program of the Institute: The results will provide clinicians with an indication of the carcinogenic potential of various chemotherapeutic agents and related chemicals in rats and mice including exposure-risk relationships, site of action, and to some extent the latency period--thereby providing a stronger basis than is now available for deciding the usefulness and manner of application of an agent suspected to be carcinogenic in man.

Proposed Course: To continue assaying the carcinogenic potential of those agents to which man is currently exposed or may be exposed for extended periods of time. Those compounds showing an increased tumorigenesis will be further evaluated for carcinogenic potential at various lengths of exposure.

Date Contract Initiated: June 24, 1968

Current Annual Level: $310,992

STANFORD RESEARCH INSTITUTE (NO1-CP-43272)

Title: Combined Effects of Chemical Carcinogens and Other Chemicals

Contractor's Project Director: Dr. David Jones

Project Officers (NCI): Dr. Robert A. Squire
Dr. Norbert P. Page

Objectives: To investigate (1) the effect of mixtures of carcinogenic and noncarcinogenic chemicals on the induction of tumors when introduced into rodents by oral administration, (2) whether the interactions among groups of chemicals in acute and subacute toxicity experiments predict the effects of the same combinations in carcinogenicity experiments, and (3) the feasibility of conducting initial screening bioassays of chemicals in groups without thereby obscuring the carcinogenicity of any individual member of the groups.

Major Findings: Thirteen compounds are being tested in both sexes of the Fischer strain 344 rat by chronic feeding singly and in various paired combinations. Each compound has been tested for identity, purity and stability in diet, and preliminary acute (2-week) and subacute (8-week) studies have been completed. Chronic feeding of them is in progress. Animals receiving Group 1 chemicals (N-methyl-N'-nitro-N-nitrosoguanidine, N-butanol-N-butylnitrosamine, nitrilotriacetic acid, dipentylnitrosamine) are at 75-80 weeks (1/10/74); mortality has been 29%; and 87% of decedents have had tumors at necropsy, principally in the appropriate target organs for these compounds. Group 2 (aflatoxin B_1, cycad flour, lasiocarpine, dipentylnitrosamine) animals are

at 21-27 weeks, and sub-chronic mortality has begun to occur in combination-fed groups receiving the high level (8000 ppm) of cycad flour. No symptoms are as yet apparent in comparable singly-fed groups. Group 3 (aflatoxin B_1, N-butanol-N-butylnitrosamine, lead acetate, thiouracil) animals are at 69-75 weeks; mortality has been 12%; and 46% of decedents have had tumors at necropsy, principally in one of the target organs (bladder). Group 4 (aflatoxin B_1, dieldrin, hexachlorophene, Aroclor 1254) animals are at 63-69 weeks, mortality has been only 4.5%; and 11% of decedents have had tumors at necropsy, most of them in nontarget organs. Mortality has averaged 3% among vehicle control groups, with a tumor incidence of 33%.

Significance to Biomedical Research and the Program of the Institute: A major portion of the resources of the National Cancer Institute is devoted to testing environmental chemicals for carcinogenicity. In these test, the experimental protocols generally attempt to expose animals to one chemical while minimizing exposure to other chemicals. There are two problems involved in this approach. First, in the human environment, men are exposed to multiple chemicals simultaneously and sequentially rather than to a single chemical. Second, resources are available to test only a small portion of the chemicals in our environment if they are tested singly. Newer chemicals are generated faster than the available resources are capable of testing each one by a protocol considered acceptable. This project is investigating how mixtures of carcinogenic and non-carcinogenic chemicals interact to affect the induction of tumors and also is investigating the feasibility of conducting initial screening bioassays of chemicals in groups without thereby obscuring the carcinogenicity of any individual member of the group. The biological studies from this contract will be analyzed by mathematical statisticians working on a separate contract with the NCI to determine whether consistent mathematical models of interactions can be developed.

Proposed Course: It is estimated that this project will extend over four years. Acute and subacute studies of individual chemicals and pairs of chemicals were conducted in order to determine the dosage levels to be administered in the chronic carcinogenic studies. The results of all three studies will be analyzed statistically in order to determine what extent the interactions of two chemicals in carcinogenesis parallel the interaction seen in the short-term acute and subacute studies.

Date Contract Initiated: June 24, 1971

Current Annual Level: $978,616

TEMPLE UNIVERSITY (SKIN AND CANCER HOSPITAL) (NO1-CP-43271)

Title: UV Carcinogenesis: Effects of Depletion of Atmospheric Ozone

Contractor's Project Director: Dr. Paul D. Forbes

Project Officer (NCI): Dr. Norbert P. Page

Objectives: The primary objective of the proposed investigation is to identify and quantitatively assess the relationship between changes in the "effective thickness" of stratospheric ozone and the development of solar damage to the skin, particularly carcinogenesis. The initial experiment is designed to give data based on six effective thicknesses of ozone, the principal biologic endpoint being carcinogenesis in the skin of hairless mice. A xenon arc lamp will be used to simulate sunlight, and an optical filtration system will be used to simulate changes in the stratospheric ozone content. The primary question to which this proposal is addressed is one which involves the potential impact of solar energy on human skin, particularly as this interaction is influenced by changes in the quality and intensity of the ultraviolet portion of the spectrum.

Major Findings: As this is a new contract, no major findings can be reported at this time.

Significance to Biomedical Research and the Program of the Institute: In addition to providing data of immediate concern to the Department of Transportation's Climatic Impact Assessment Program, this project will yield scientific data relevant to the broad field of cancer biology. The study is designed to show whether skin cancer incidence is modified as a direct function of changes in "sunburn" UV only, while other portions of the solar spectrum are kept constant.

Proposed Course: Design, construction, and acquisition of special equipment will be completed by August, 1974, and animal exposures will begin by September, 1974. The first of the tumor data should be available in 1975. The work on this experiment is scheduled to be completed early in 1976.

For purposes of laboratory testing, the skin of hairless mice will be the biologic test material, and a xenon arc lamp will generate a simulated solar spectrum. Finding a filter with the sharp cut off characteristic of ozone is a problem for which there is very little technical background. Consequently, we are proposing to pass the light through quartz vessels containing several "effective thicknesses" of ozone. The use of a solar simulator is based upon the following reasoning: 1) there now exists a large body of evidence that the most common skin cancers of man are due to prolonged exposure to sunlight, 2) the same spectral band that causes erythema in man and rodents can produce skin cancer in mice, but the relative effectiveness of different wavelengths is unknown, 3) there is increasing evidence that long-wave UV can play a very significant role in carcinogenesis. The change in stratospheric ozone concentration will significantly modify only the short wave UV component. It does not appear possible to predict the magnitude of the effects that a change in ozone will have on the rate of carcinogenesis when one portion of the UV remains constant and another is altered. In view of these uncertainties and considering the time in which useful data must be acquired, it appears most appropriate to simulate the natural situation as closely as possible.

Date Contract Initiated: February 15, 1974

Current Annual Level: $156,704

TRACOR JITCO, INCORPORATED (NO1-CP-43350)

Title: Bioassay Operations Prime Contract

Contractor's Project Director: Dr. Carl J. Wessel

Project Officer (NCI): Dr. Norbert P. Page

Objectives: (1) Assume prime contractor responsibility for nine contracts now funded by the Bioassay Operations Segment; (2) Assume coordinating responsibility for all other contracts in the Bioassay Operations Segment or as assigned by the NCI project officer; (3) Develop a management system to coordinate, monitor, and review the carcinogenicity testing program assuring adherence to specified performance standards; (4) Obtain working knowledge and familiarity of entire program, study existing Bioassay Operations System, protocols in use, management methods, and propose refinements to improve the NCI bioassay operations; (5) Coordinate animal and chemical resources and their distribution to testing laboratories; (6) Assure safety of contractor personnel in handling chemicals of unknown toxicity or carcinogenic activity; (7) Maintain inventory of program resources, expenditures of funds, and anticipated budget projections; (8) Provide for data submission to the Carcinogenesis Bioassay Data System. Provide additional information to the NCI where a chemical file is maintained containing information on chemicals which have been tested or are currently being tested, basic protocols in use, and other selected data elements; and (9) Organize site visits, program reviews, provide for data analysis, prepare reports in response to specific needs of the prime contractor or NCI project officer.

Major Findings: No findings can be reported, as this contract was initiated on March 1, 1974.

Significance to Biomedical Research and the Program of the Institute: The National Cancer Act of 1971 provides legislative authority for the National Cancer Institute to plan and develop an expanded, intensified, and coordinated cancer research program, encompassing the programs of the National Cancer Institute, related programs of the other research institutes, and other Federal and non-federal programs. Protecting the population from chemical and physical carcinogenic hazards and their effects has been clearly recognized as an urgent and essential task in our society, and a high national priority for this mission has been forcefully indicated by the new Cancer Act, by Congress, and by the Administration. The Carcinogenesis Program of the Division of Cancer Cause and Prevention has been developed to serve towards the accomplishment of these national goals. One of the major program activities is the identification of carcinogenic activity of selected agents by bioassay testing. This testing program is managed by the Bioassay Operations Segment of the Carcinogenesis Collaborative Contract Program.

This project will provide prime contract support to the Bioassay Operations Segment in administering and coordinating this important national program. It is anticipated that the contracting for management and scientific capabilities will allow the NCI scientific staff to devote its attention more to program

development and expansion of the carcinogen bioassay testing programs.

Proposed Course: In the first year, the prime contractor will assume responsibility for nine existing contracts with a phase-in of one per month. As the contractor gains an understanding of the program more scientific responsibilities will be assumed.

Date Contract Initiated: March 1, 1974

Current Annual Level: $3,724,222

WOLF RESEARCH AND DEVELOPMENT CORPORATION (NO1-CP-23302)

Title: Information Systems Microfilm Services to Carcinogenesis Bioassay Data System (CBDS)

Contractor's Project Director: Mr. Dalton Tidwell

Project Officer (NCI): Mr. Terence Kuch

Objectives: This project links data and information among the CBDS bioassay contractors, the Bioassay Operations Segment, the Carcinogen Bioassay and Program Resources Branch, and the CBDS computer subsystem implemented through the Data Management Branch, Division of Computer Research and Technology. The contractor operates a subsystem to accept, log, and process data collection forms submitted by the scientific research organizations participating in the CBDS. He provides trained and qualified staff to operate this subsystem with functions including: forms control, editing and coding, maintenance and development of a microfilmed data base; operation of an animal inventory; and maintenance of automated files for contract information and chemical information.

Major Findings: An operational system to control and process CBDS bioassay contractor data from initial data entry to updating the CBDS data base and creating microfiche records for all data processed has been developed and documented in collaboration with Program and with DCRT, NIH. A CBDS Chemical File has been developed to maintain current chemical information for all chemicals on bioassay testing. A CBDS Contract File has been developed to display administrative, chemical, and treatment information for all agents being tested by bioassay contractors. As an ongoing effort, support is provided in a collaborative study with the International Agency for Research on Cancer (IARC) Registry program. Information on chemicals being tested for carcinogenicity, under NCI contract, is being compiled and routinely submitted to IARC, Lyon, France. During the current contract period, over 63,000 data collection forms were processed and entered into the CBDS data base and recorded on microfiche. These records are available for analysis by NCI investigators and others by special arrangement.

Significance to Biomedical Research and the Program of the Institute: Data collected from bioassay studies are important in formulating public policy

pertaining to the continued use of the agents tested. It is important that the results of these studies be properly recorded and analyzed. The CBDS will provide these functions as it develops.

Proposed Course: The subsystem implemented by the contractor is functioning and is proposed to continue in operation during the data collection phase of the carcinogenesis bioassay study.

Date Contract Initiated: June 26, 1972

Current Annual Level: $152,662

SUMMARY REPORT

BIOLOGICAL MODELS SEGMENT

July 1, 1973 through June 30, 1974

The Biological Models Segment was established in the fall of 1971 and charged with the responsibility for developing experimental models for studying the etiology and pathogenesis of the major forms of human cancer and utilizing these models for developing methods to prevent or modify the development of human cancer. Separate segments exist for lung cancer and cancer of the colon and rectum, but the remaining types of human malignancy fall within the area of responsibility of the Biological Models Segment.

The Segment program presently includes a total of 28 contracts covering the following areas of carcinogenesis studies:

a. Pancreas
b. Prostate
c. Mammary Gland
d. Perinatal carcinogenesis
e. Other (liver, kidney, chalones, etc.)

Each of these sub-programs are described below. Within the interpretation of this Segment's goals, model development is not limited to use of whole animals, though this is perhaps the greatest emphasis of the program. Cell and organ culture studies are specifically designed to develop the appropriate methodology for long-term maintenance of tissue in as near a normal state as possible, as a tool for defining early stages of the carcinogenic response.

A number of personnel changes have taken place this year. With regret, the Segment observed the reassignment of Dr. Richard R. Bates as Scientific Coordinator for Carcinogenesis at the NCI's Frederick Cancer Research Center. With his new laboratory in Frederick, it became necessary for him to resign his position as Director of the Biological Models Segment. However, the activity of the program is fortunate in having Dr. Bates maintaining an interest in and being a working member of the Segment. The former duties of Dr. Bates in the Segment have been assigned to Dr. Richard A. Pledger, serving as Acting Director and Manager.

The expertise of the Segment's staff was greatly enhanced by the addition of Dr. Robert A. Squire to the Segment's membership. Dr. Squire is the Head of the Tumor Pathology Section of the Carcinogenesis Program and will provide significant input to the growth, planning, and implementation of the Segment's program.

Pancreas Carcinogenesis

This Segment applied its greatest effort to the initiation of a pancreas carcinogenesis program in 1971. The dearth of knowledge concerning all

aspects of pancreatic cancer, the rising incidence of this disease in the American population, and the little being done to develop a systematic approach to understanding its cause and prevention justified the efforts made by the advisory group in collaboration with the Staff in stimulating research within this area. Currently, eight contracts are supported, investigating cell and organ culture of exocrine pancreatic cells and specific approaches to induce adenocarcinoma in laboratory animal species.

During this past year a number of scientific observations were made within the program which have significant impact on the future direction of the program. Within 36 weeks of initiation of weekly administrations of methylnitrosurea, pancreatic adenocarcinoma has been observed in strain 13 guinea pigs at a 36% incidence level. This rapid induction, demonstrated by Dr. J. Reddy at the University of Kansas Medical Center, as compared to the 800-day figure reported by Druckery, provides a more effective tool to investigate the early aspects of carcinogenesis. Future activities will clearly be directed toward determining the age of 100% incidence and developing ways to further influence incidence and latency of this induction. One interesting observation is the apparent genetic influence of the guinea pig strain, inasmuch as other strains have not responded as yet to a similar protocol.

The pancreas sub-program is unique in that planning sessions by the NCI Staff and advisors are supplemented by the collaborative investigators themselves. The pancreas program on cancer supported by the Segment is the only known one of its kind in existence. Periodically, a special one day workshop is held, composed of all interested investigators and staff, to discuss the current level of program accomplishment and to make suggestions and recommendations for future project implementation. This participatory mechanism can only strengthen the attainment of the Segment's goals.

The Carcinogenesis Program has recently awarded a contract which shall attempt once and for all to resolve the disagreement as to whether the acinar or ductal cell of the pancreas is the principal site of origin of this disease. In addition, the talent base is being expanded by the planned award of a Research and Management Contract by which additional expertise will be developed and secured for implementation of the program.

Prostate Carcinogenesis

A program similarly initiated in 1971 and consisting of seven projects is that of prostate carcinogenesis. During the past year, observations by Dr. S. Shain at Southwest Foundation for Research and Education have offered a new direction in research of this disease. Significant differences between young and old normal, untreated animals have been observed in certain biochemical and physical parameters of the rat prostate. As rats mature, there is a reduction in the capacity for testosterone metabolism and a shift to oxidative schemes primarily

manifest as an accumulation of 4-androstenedione (a metabolite absent from young tissues), as well as a progressive decrease in the ratio of 17-OH to 17-keto metabolites. Moreover, assessment of the high affinity, androgen-binding sites in the ventral prostate shows a strong likelihood of a decreased number of sites per cell as the rat matures. With the few 11-12 month old animals evaluated, the value for binding sites has been reduced to 5-30% of the young 2-5 month old animal. Inasmuch as prostate cancer is a phenomenon principally of old age and that the incidence and change from latent to active carcinoma increases with age, it is probable that the aging process itself can significantly influence prostate carcinogenesis. Further work will clearly require colonies of aged animals in order to develop a greater understanding of the aging process, as well as utilizing such aged animals for induction studies using carcinogens.

Mammary Gland Carcinogenesis

The mammary gland program consists of four projects oriented toward model development. Success has been achieved in demonstrating nodular "transformation" of mammary epithelium by a chemical carcinogen within 2-3 weeks in organ culture. Dr. M. Banerjee of The University of Nebraska has concluded that histologically the alveolar nodule-like structures induced in organ culture in his laboratory are indistinguishable from the precancerous, hyperplastic mammary nodules induced in the intact animal by virus, chemical carcinogens, or hormones. Most interesting is the technique used, a hormone-deficient medium in which the normal hormone-dependent alveolar structures regressed, permitting visualization of the "altered" hormone-independent structures. This altered hormone requirement also characterizes the in vivo precancerous mammary nodules. Once this system is refined, it will be possible to begin studying the initial cellular changes that lead to the visible nodular lesion described.

Perinatal Carcinogenesis

Another program area receiving particular attention and expansion is that of perinatal carcinogenesis. Two projects are currently supported; however a number of new approaches will be initiated shortly. As a result of a meeting held in Tokyo, Japan, it has become clear that certain hormones and other stimuli on the developing fetus can have significant influence on adult physiology, including the incidence of cancer. Current evidence clearly links exposure of the human fetus to abnormal estrogen levels to development of vaginal cancer two decades later in life. Major projects will be initiated which will identify a cellular basis for long-term changes to mammalian target organs after perinatal exposure to hormones. These studies would have implication not only on cancer in general but also childhood tumors that most likely have their genesis during the fetal or perinatal stage of development. In addition, an emphasis was placed on determination of the mechanism responsible for the increased susceptibility of fetal and neonate tissue to carcinogenesis. Such studies as these will be initiated to provide

insight in model development as well as the goal of developing means to prevent the induction of cancer.

Others

A few specific approaches are supported in the study of chalones and in liver and kidney carcinogenesis, principally in support of research opportunities that have been presented which lead to a greater understanding of animal model development. Dr. Farber at Temple University has recently observed the presence of a protein antigen which seems to be a highly specific marker for hepatocytes in hyperplastic nodules in liver during hepatocarcinogenesis induced by a variety of carcinogens. This could significantly offer a useful tool for studies of the process of liver cancer induction. An animal model for kidney cancer is under development at the University of Maryland and is currently being evaluated in comparative studies with human kidney lesion development and progression. Chalones, a tissue specific inhibitor of mitosis, is being purified for eventual studies in its potential regulatory control of growth and its usefulness for possible treatment of existing neoplastic lesions.

The Biological Models Segment has sponsored a number of workshops within which to identify program areas of high priority and relevance. As a result of these, one prime area which shall be considered for program initiation during the next fiscal year is that of the effect of biological rhythms on cancer. It is already well known that chemotherapeutic approaches to disease modification are significantly influenced by the time of day certain drugs are administered. Preliminary evidence is available to suggest that infradian, circadian, or ultradian cycles can influence the carcinogenesis process in laboratory animals. Efforts will be undertaken to extend these studies as relevant approaches are identified.

The advisory group associated with this Segment consisting of recognized scientists from universities thoughout the country has been relatively stable, having only one member resign for purposes of avoiding the appearance of conflict of interest. The existing advisory group members have been associated with the Segment from its inception in 1971. This has provided not only their individual expertise in advising the Segment but a stability which offers a total initiation, implementation, and review cycle consistent from one year to the next. Their input and value cannot be quantitatively expressed, but the results of their interaction are appreciated by the NCI Staff and can be inferred from the positive and scientifically significant results obtained during the relatively short period of their service.

Interaction continues with similar program groups such as The American Pancreatic Study Group, National Prostatic Cancer Project, Breast Cancer Program, The National Bladder Cancer Project, and The National Organ Site Programs Branch either by direct, periodic contact or with staff and advisory group members occupying a liaison role for the Segment. It is through such contact that each group can augment relevant studies based upon immediate assessment of the implication of results of another.

CONTRACT NARRATIVES

BIOLOGICAL MODELS SEGMENT

July 1, 1973 through June 30, 1974

ALTON OCHSNER MEDICAL FOUNDATION (NO1-CP-43229)

Title: Carcinogenesis by Radiation plus Estrogen

Contractor's Project Director: Dr. Albert Segaloff

Project Officers (NCI): Dr. Norbert Page
Dr. D. Jane Taylor

Objectives: (1) To investigate interaction or synergism of radiation and hormones in the induction of mammary tumors. (2) To evaluate the feasibility of establishing an artificial estrus cycle which can be maintained for a prolonged period of time.

Major Findings: The contractor has been able to show that a single dose of x-rays delivered to one mammary chain of AxC inbred rats which had also received a continuous exogenous source of diethylstilbestrol (DES) resulted in a significant increase in mammary tumors over that resulting from the estrogen alone. Some of the non-estrogen treated control animals which were radiated have started to develop tumors. It is of interest that these early radiation-induced mammary tumors are frequently benign as opposed to the consistent malignant nature of the tumors produced in the mammary gland of DES-treated or DES-radiation-treated animals. When early mammary tumors alone are considered, there is a dosage response between radiation in the range used (50, 150 or 450 r) and tumor induction in the DES-treated rats. However, when the total number of tumors induced is considered, the greatest synergism is found with the median dose employed, 150 r. Although preliminary evidence in female AxC rats indicates that continuous administration of progesterone will inhibit both estrogen-induced mammary cancer and radiation (800 r)/estrogen synergism, the evidence developed under this contract in castrated female AxC rats suggests that these animals have a much lower incidence of DES-induced mammary cancers with a longer latent period. Further, neither the continuous nor cyclic administration of progesterone inhibits the DES induction of mammary cancers in the absence of the ovaries.

It has been found that the induced mammary cancers contain many mast cells. It is believed that data analysis will show that their numbers and distribution will correlate with tumor growth rates. Using electron microscopy the Special Virus Cancer Program has been unable to demonstrate virus particles in the induced tumors.

Studies are underway to ascertain if the diethylstilbestrol itself or its induced high level of serum prolactin or both are necessary at the time of radiation for the optimal expression of the synergism. To date it has been demonstrated that the shortest latent period is associated with the presence

of diethylstilbestrol and an elevated serum prolactin at the time of radiation. A modest delay in the latent period of carcinogenesis is seen when neither diethylstilbestrol nor the increased prolactin is present at the time of radiation. The greatest increase in latent period seen to date has been when the elevated prolactin is present at the time of radiation but the diethylstilbestrol is present only starting two weeks after the radiation.

Significance to Biomedical Research and the Program of the Institute: Both radiation and ovarian hormones, given individually in large doses, have been implicated in causing an increase in breast cancer of both laboratory animals and women. It has been speculated that x-rays, routinely used for diagnosis and radiotherapy, plus estrogens and progesterone, now being widely used in birth control or for postmenopausal therapy, may interact synergistically and be contributing to the alarming rise in the incidence of breast cancer.

In recent years the Carcinogenesis Program of NCI has devoted some efforts and funds to studies of the hormones, progestogens and estrogens which are often combined in contraceptive pills. This study is an extension of efforts in this field.

Proposed Course: The radiation/estrogen studies now underway are expected to be completed by mid-1975. Additional studies are being considered to vary dosages of estrogen administered by various routes which would mimic more closely the estrogen content in the human female contraceptives.

It is also planned to continue research to set up an artificial estrus cycle of the same length as the natural cycle of the rat. The point of major importance here would be to determine the dosage of estrogen (estradiol-17β, ethinylestradiol, etc.) necessary to duplicate the natural estrus cycle in order to determine precisely the quantity of estrogens required (with the additional amount found in the contraceptives) to influence the induction of mammary tumors in combination with a known dosage of x-ray.

Various experiments are underway attempting to establish the nature of the ovarian contribution to both the synergism and the fundamental carcinogenesis mentioned above. This could prove to be another steroid such as the androgen dehydroepiandrosterone or a nonsteroidal component of ovarian secretion such as relaxin.

Studies are also underway to ascertain if it is possible to induce mammary cancer with DES alone or in synergism with radiation in the absence of the animal's pituitary. Hypophysectomized animals are delicate and their long-term maintenance is difficult. A major effect may be the same as that seen for oophorectomy, namely, a doubling of the latent period for carcinogenesis. This is particularly possible since the removal of the pituitary also leads to hypofunction of the ovary.

Studies are now being initiated for the extension of the radiation-estrogen work to the Fischer 344 strain of inbred rats. The 150 r dose of radiation is being employed. Since the Fischer rat strain is less long-lived than the AxC strain and is at a somewhat higher risk of spontaneous mammary tumors than the AxC strain, this data will be most important in evaluating the human disease.

Date Contract Initiated: May 25, 1971

Current Annual Level: $120,045

BOSTON UNIVERSITY (NO1-CP-23297)

Title: Controlled Methods for the Delivery of Chemical Carcinogens to the Pancreas

Contractor's Project Director: Dr. Richard Elkort

Project Officer (NCI): Dr. Richard Pledger

Objectives: To attempt induction of adenocarcinoma of the pancreatic duct by surgical implantation of a permeation chamber containing chemical carcinogens.

Major Findings: Observations, up to 52 weeks post-implantation, confirmed that both the control and carcinogen containing intra-pancreatic permeation catheters are well tolerated by the host animal for long periods of time. Two carcinogens are currently being evaluated. These are, 40% dimethylhydrazine and benzo(a)pyrene. Control animals in both groups implanted with catheters containing carcinogen solvent only, show normal pancreatic morphology after the initial reactive phase has passed. Rabbits implanted with catheters containing 40% dimethylhydrazine show pancreatic ductal cell hyperplasia, metaplasia and dysplasia with periductal cell infiltration and some disorganization of the epithelium lining the ducts. These changes begin approximately nine to twelve weeks post-implantation and persist until 32 to 40 weeks post-implantation. Silicone polycarbonate catheters with a 4 to 10 day carcinogen release rate were used initially. More recent experiments have been done utilizing nylon catheters with a more prolonged release rate. Observations in this latter group have only been made up to 10 weeks post-implantation. A small number of animals show the ductal changes previously noted in the silicone catheter DMH group and manifest widespread acinar hyperplasia and dysplasia as well. Observations in the benzo(a)pyrene group of animals up to 26 weeks post-implantation have shown progressive pancreatic regeneration after an initial phase of acinar atrophy induced by the carcinogen. The possible relationship of this regeneration to subsequent neoplasia is being examined. By substituting nylon for silicone polycarbonate catheters in the benzo(a)-pyrene experimental group, it has been possible to obtain a 30 to 40-day carcinogen release rate compared with the 4 to 10-day rate previously achievable with the silicone polycarbonate system.

Specificity of the delivery systems that are being utilized appears to be excellent in that effects are observed on the pancreatic and peripancreatic tissues only. Moreover, these effects are probably directly related to the implanted carcinogens since similar effects are not observed in the control animals.

Significance to Biomedical Research and the Program of the Institute: The establishment of an animal model for pancreatic adenocarcinoma of man is a prime goal of the Carcinogenesis Program. This method represents only one

approach being used. If induction occurs by physically implanting the carcinogen at the target cells of interest, a detailed study of the neoplastic process may then subsequently be made.

Proposed Course: Contractor will continue to determine the long-term effects of carcinogen exposure by observations in each carcinogen group up to two years post-implantation. Implantation of the "slow release" nylon permeation catheters will be completed in additional groups of animals to maximize the duration of carcinogen exposure. Pilot studies will be undertaken to determine the feasibility of implanting exteriorized intra-pancreatic permeation catheters which can be periodically recharged with carcinogen so as to make extremely prolonged exposure with chemically unstable carcinogens possible. Finally, consideration will be given to the evaluation of additional "direct acting" carcinogens to the agents already being tested in this study. A subcontractor (Abcor, Inc.) will continue to supply the catheter permeation devices and will continue to provide in vitro data relating to carcinogen release rates, carcinogen analytic techniques and determination of residual carcinogen in the catheter at the time of animal sacrifice.

Date Contract Initiated: June 28, 1972

Current Annual Level: $58,916

BROOKHAVEN NATIONAL LABORATORY (YO1-CP-33329)

Title: Synergistic Interaction of Hormones and Neutron Radiation on Mammary Gland Carcinogenesis

Contractor's Project Director: Dr. Claire J. Shellabarger

Project Officers (NCI): Dr. Norbert Page
Dr. Douglas Janss

Objectives: To determine if the synergistic interaction between x-irradiation and estrogen on mammary gland carcinogenesis in the female AxC rat, as observed by Segaloff, holds also for neutron-irradiation in female AxC rats and in female Sprague-Dawley rats.

Major Findings: Data from two pilot experiments, started before the contract was initiated, show that either 320 or 350 days after DMBA treatment AxC female rats give much smaller mammary adenocarcinoma and fibroadenoma responses than do female Sprague-Dawley rats. Another pilot experiment shows that one year after either neutron- or x-irradiation female AxC rats have a lower incidence of mammary neoplasia than do female Sprague-Dawley rats. These results, to be used in planning additional experiments, suggest that the mammary neoplastic response of the AxC strain to physical or chemical carcinogens is either smaller/and or has a longer latent period than the Sprague-Dawley strain.

Two experiments have been started but it is too soon to have any tumor results. In one, female AxC rats were given neutron-irradiation with and without DES. In the other, female Sprague-Dawley rats were given either neutron- or

x-irradiation, with and without DES or progesterone.

Significance to Biomedical Research and the Program of the Institute: It has been reported (Segaloff) that x-radiation and DES interact in a synergistic manner in the induction of mammary adenocarcinoma in female rats of the AxC strain. Other studies (Vogel,Shellabarger) have indicated a large relative biological effectiveness (RBE) for neutron-irradiation in the induction of mammary neoplasia in female rats of the Sprague-Dawley strain. Because it has been reported that women exposed to repeated fluoroscopic examinations, women survivors of atomic bombings, and possibly women treated with x-ray for acute postpartum mastitis, all have an increased risk of developing breast cancer, radiation exposure is now associated with breast cancer of the human female. With the more frequent use of neutron services in industry, research, and medicine, the possibility of increased exposure of women to neutron-irradiation is expanded. Also, the use of sex steroids for contraceptive and/or replacement therapy has increased. Thus, the interactions of neutron-irradiation and sex steroids in regard to mammary carcinogenesis should be evaluated. This project is an attempt to develop an animal model, and to understand the model itself, so that the qualitative aspects of mammary carcinogenesis in man may be evaluated in terms of cause and prevention of breast cancer.

Proposed Course: Initially, attempts will be made to answer the neutron-hormonal interaction question by repeating the Segaloff experiments in female AxC rats substituting neutron-irradiation for x-irradiation.

Additional experiments will be set up using females of the Sprague-Dawley strain in order to compare radiation-hormonal interaction on mammary carcinogenesis in the 2 strains. This comparison should give a general indication of whether or not the synergistic interaction between x-irradiation and estrogen observed in the AxC strain can be extended to neutron-irradiation in the AxC strain and the Sprague-Dawley strain.

Date Contract Initiated: June 28, 1973

Current Annual Level: $193,000

CASE WESTERN RESERVE UNIVERSITY (NO1-CP-23284)

Title: Enhanced Induction of Guinea Pig Pancreatic Adenocarcinoma

Contractor's Project Director: Dr. Samuel S. Epstein

Project Officer (NCI): Dr. Richard Pledger

Objectives: This project is designed to confirm earlier reports on induction by nitrosamides of exocrine pancreatic adenocarcinoma in guinea pigs, to explore methods for attempting to enhance the incidence and decrease the latency of these tumors, and to investigate the biochemical mechanisms involve in pancreatic carcinogenesis.

Major Findings: (1) Alkylation of maternal pancreatic guinea pig DNA or RNA has been demonstrated both in vivo and in vitro with NMU and NMUT. NMUT is a more potent alkylating agent than NMU; this may be relevant to the greater carcinogenic potency of NMU in the guinea pig pancreas. It has also been shown that NMU and NMUT alkylates adult and fetal liver and brain DNA and RNA in vivo with the formation of 7-methyl guanine.

Uptake of labelled NMU and NMUT appears greater in the duodenal and middle segments of the pancreas than in the splenic segment. Four hours following gavage, the uptake of labelled carcinogens was highest in the liver, with lower and similar levels in the pancreas, kidney, stomach, and brain.

(2) A method has been devised to reproducibly measure DNA synthesis, in the guinea pig pancreas in vivo, by the use of intracardiac injections of ^{3}H-thymidine. Preliminary autoradiographic data indicate that partially pancreatectomized animals have a much higher labeling index in their acinar cells than in the case with control or sham operated animals.

(3) The transplacental carcinogenic effects of NMU and NMUT have been investigated, using approximately 426 guinea pigs dosed 3 times weekly by gavage with NMU (2.5 mg/kg) or NMUT (1.25 mg/kg) during the latter part of pregnancy; this study has yielded approximately 900 F_1 progeny, which are now being observed for the duration of their lives.

Some maternal and F_1 animals have been subject to partial pancreatectomy in attempts to investigate whether regenerating cells have an enhanced susceptibility to chemical carcinogens. The incidence of stillbirths, neonatal and perinatal deaths, and weight loss is higher in NMU than NMUT groups. Diffuse hepatic necrosis and fatty degeneration has been found in some F_1 progeny of NMU treated females.

(4) Carcinogenesis studies of Hartley Strain adult males have been initiated. These are based on approximately 300 adult males, dosed 3 times weekly by gavage with NMU (2.5 mg/kg) or NMUT (1.25 mg/kg). Some groups have been subject to partial pancreatectomy. Significant weight loss and mortality has been observed in the NMU groups, and all dosing with NMU has for this reason been discontinued after approximately 6 months; however, hepatic necrosis and fatty degeneration has been found in some NMU treated adults. One animal on NMU has developed a pancreatic tumor 360 days following treatment.

The strain 13 female study is based on 70 adult female guinea pigs, gavaged twice weekly with NMUT (1.25 mg/kg). Some groups have been subjected to partial pancreatectomy. No weight loss or mortality has been observed in any group.

Significance to Biomedical Research and the Program of the Institute: The elucidation of the mechanisms of induction of adenocarcinoma of the pancreas in the guinea pig is likely to aid in the determination of the biological factors critically involved in the pathogenesis of human pancreatic cancer. Factors such as transplacental exposure; increased cell division and effects of environmental pollutants, such as asbestos which has been found in the pancreas of humans dying of asbestosis or mesotheliomas in relatively high

concentrations, will also be investigated, with particular reference to the possibility of in vivo interactions with nitrosamide carcinogens.

Proposed Course: Future activities will involve determination whether an enhanced incidence of pancreatic adenocarcinoma results from transplacental exposure of guinea pigs to NMU and NMUT. Similarly, the effects of enhanced cell division (induced by partial pancreatectomy) will be studied on maternal animals and on their F_1 progeny. Additionally, the following investigations will be undertaken: (a) the effects of NMU and NMUT on DNA and RNA synthesis in the Hartley Strain, as well as in the Strain 13, guinea pigs; (b) comparative uptake of NMU and NMUT in the Hartley as well as the Strain 13 guinea pig pancreas; (c) a study of the autoradiographic localization of ^3H - NMU and NMUT in the Hartley guinea pig pancreas and following partial pancreatectomy, as well as in the Strain 13 guinea pig pancreas; (d) studies on the pathogenesis of liver lesions induced by NMU, following transplacental administration and following administration to adult animals.

Date Contract Initiated: June 29, 1972

Current Annual Level: $98,000

CHICAGO, UNIVERSITY OF (NO1-CP-33287)

Title: Definition of Sensitivity of Carcinogenesis Bioassay Systems

Contractor's Project Director: Dr. Stan Vesselinovitch

Project Officer (NCI): Dr. Jerry Rice

Objectives: To establish standard bioassay systems in mice for screening substances suspected of potential carcinogenic hazard to man, making best use of the age-dependent variation in sensitivity to chemical carcinogens exhibited by F-1 hybrid mice (first generation crosses of C3HeB x A/He and C57Bl/6 x C3HeB) and to provide definition of diagnostic criteria and of detailed biologic behavior of the neoplasms produced, especially those of the liver.

Major Findings: Initial studies on the sensitivity to various carcinogens of both hybrid strains of mice have been concluded, and are being prepared for publication. The appearance in these mice of large numbers of renal epithelial tumors, which are uncommon in this species, has allowed a study of the morphogenesis of these tumors. Studies of continuous pre- and postnatal exposure of hybrid mice to known human carcinogens, which require enzyme-mediated conversion to reactive metabolites, is nearing completion and should indicate the feasibility of including perinatal exposure in the usual carcinoge screening programs in order to increase the sensitivity of such tests. Transplantation studies with hepatomas induced by different agents have revealed that a massive, hyperplastic reaction to amitrol is generally not neoplastic and serves as a model for the type of reactive lesion which must be distinguish ed from neoplasia. Careful search of the lungs of mice which have developed liver tumors in response to various carcinogens has revealed a significant incidence of metastasis, confirming the malignant nature of these tumors.

Significance to Biomedical Research and the Program of the Institute: A major deficiency in carcinogen screening programs has been their lack of uniformity. It has been, in the past, quite impossible to estimate the relative carcinogenic potency of different compounds, even those tested in the same species, because of uncontrolled variables associated with testing protocols. These include the intrinsic differences in neoplastic response of different tissues in various strains of mice, the pronounced quantitative differences in response of fetuses, infants, and adults, and the marked effects of route of administration and dosage regimen. The dissimilarity between the histological features of murine versus human tumors of corresponding cell type has also led to disagreements, which require resolution, concerning the significance of certain neoplasms of the mouse to assessment of cancer risk in man.

The purpose of this contract is to evaluate the significance of these variables within the context of an optimal screening protocol, and to develop improved standardized procedures which will be used for future carcinogen screening programs. Definition of biologic behavior and diagnostic criteria for the histologic identification of the neoplasms that can be induced in these animals will provide needed reference standards for the evaluation of endpoints in carcinogenesis tests.

Proposed Course: Studies presently underway will be concluded in an orderly fashion and the results prepared for publication over the next 18 months.

Date Contract Initiated: June 25, 1969

Current Annual Level: $294,430

CHICAGO, UNIVERSITY OF (NO1-CP-33376)

Title: Route of Carcinogen Administration in Pancreatic Adenocarcinoma Induction

Contractor's Project Director: Dr. Stan D. Vesselinovitch

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: The objective of this project is to explore several animal species and various routes of administration of carcinogens in an effort to enhance the carcinogenic dose to the pancreas or the susceptibility of the target tissue.

Major Findings: Methylnitrosourethan (MNUT), 4-hydroxyamino quinoline-1-oxide (HAQO), benzo(a)pyrene (BP) and 2,7-fluorenylene bisacetamide (AAF) has been administered by intravenous, intraperitoneal, orally, and by intra-organ inoculation into rats, mice, and dogs with variable results. All rat studies with HAQO to date (one year incubation period) have failed to demonstrate induction of pancreatic adenocarcinomas. Oral administration of MNUT has resulted in one ductal and one acinor pancreatic adenocarcinoma with

60 animals initially treated. BP administered IP or intraorgan has induced 1-3 adenocarcinomas within 27-38 weeks within a group of 60 treated animals.

Only BP was found to induce tumors of the pancreas in mice--one from oral administration, though stomach tumors were similarly found, and one from direct intraorgan inoculation. It is apparent that within 26 weeks only a minimal response is observed. Another twelve months will be required to adequately assess these protocols.

Significance to Biomedical Research and the Program of the Institute: This approach to the problem of establishing a biological model of pancreatic carcinogenesis offers potential to the Program. Until such time that a reproducible, experimental protocol is available which characteristically induces cancer of the pancreas, the research necessary to enable an understanding of the cause and ultimate prevention of this disease will be markedly restricted. Model development is one of the major goals, and this project, along with the others, is designed specifically to reach this goal.

Proposed Course: Animals will be maintained for another year during which time exposed animals will be examined periodically for evidence of tumor induction. Careful histopathological studies will be performed to determine the primary tissue involved.

Date Contract Initiated: June 28, 1972

Current Annual Level: $125,000

CHILDREN'S HOSPITAL RESEARCH FOUNDATION (NO1-CP-33307)

Title: Etiology of Medulloblastoma and other Brain Tumors

Contractor's Project Director: Dr. Josef Warkany

Project Officer (NCI): Dr. Jerry M. Rice

Objectives: (1) To investigate the relationship of congenital malformations and transplacental carcinogenesis. (2) To develop a model for induction of medulloblastoma and other brain tumors in rodents.

Major Findings: To achieve the objectives mentioned above, the contractor is combining teratogenic methods with transplacental and perinatal carcinogenic methods. Congenital malformations of the brain are produced in rats by prenatal X-irradiation which results in microencephaly, microphthalmia and other anomalies. After application of a teratogenic dose of X-rays to pregnant females, treatment with ethylnitrosourea (ENU) follows. Effects of the carcinogen on normal offspring and on malformed offspring are being compared. In mice, transplacental carcinogens are administered to dreher stock mice whose offspring have hereditary malformations of the cerebellum and to mice treated with galactoflavin which can produce malformations of the hindbrain in the offspring. At the present time, tumors appearing in malformed and in "normal" offspring of rats are being compared. Although transplacental

carcinogens given to mice have caused growth retardation, tumors have not yet appeared.

Significance to Biomedical Research and the Program of the Institute: Although models are available that permit production of brain tumors in rodents, there is a need for models that simulate childhood tumors of the central nervous system, particularly medulloblastomas. The project underway is also testing the long-standing hypothesis that congenital malformations predispose to tumors.

Proposed Course: Tumors developing in malformed and "normal" offspring will be examined and compared. Special efforts will be made to combine carcinogenic experiments with teratogenic methods which result in malformations of the cerebellum. It remains to be seen whether malformed cerebella will react specifically to transplacental or perinatal treatments with carcinogens.

Date Contract Initiated: March 27, 1973

Current Annual Level: $84,923

CORBEL LABORATORIES, INC. (NO1-CP-23299)

Title: Provide Animal Holding Facilities and Services

Contractor's Project Director: Mr. William Rickman

Project Officer (NCI): Dr. Jerry Rice

Objectives: The goal of this project is to demonstrate transplacental chemical carcinogenesis in the Old World monkey, Erythrocebus patas, as a representative primate species. It is uniquely suited for this purpose because of the readiness with which it breeds in captivity; the availability of a nucleus colony of this species; and the active participation of an experienced primatologist, Dr. William London, of the Infectious Disease Branch, National Institute of Neurological Diseases and Stroke.

Major Findings: Although importation of additional breeding monkeys has been slower than originally anticipated, the nucleus colony has been sufficient for preliminary studies of dosage. The pregnant patas tolerate single or repeated injections of 0.1 mmoles/kg of 1-ethyl-1-nitrosourea (ENU), either intravenously in pH 3 isotonic citrate buffer or intraperitoneally in trioctanoin, if administered after the 50th day of gestation (normal gestation: 160 days). Larger doses cause abortion, which may not occur until several weeks after treatment. A total of 28 monkeys exposed in utero to ENU were under observation for tumor development when it was discovered that this species is a carrier of simian hemorraghic fever (SHF), an arbovirus infection fatal to macaques. Under some conditions, SHF is pathogenic for patas also, but not for man. Continuing studies will be restricted to animals which are serologically negative for this virus. No tumors have yet been observed up to 18 months after in utero exposure to ENU.

Significance to Biomedical Research and the Program of the Institute: Cancer is the third most frequent cause of death among infants and children in the United States and most industrialized countries, taking a lesser toll than either accidents or infectious diseases, but claiming nearly as many lives as the latter. Many of the specific varieties of cancer encountered in children are found uniquely or predominately in this age group and not infrequently develop so early in life that a prenatal origin is either certain or highly probable. With the recognition of the association between exposure to diethylstilbestrol in utero and the development of vaginal adenocarcinoma during the second decade of life, the possible significance to human health of exposure to transplacental chemical carcinogens has become increasingly a matter of concern. Experimental studies on transplacental chemical carcinogenesis have been limited to rodent species which differ significantly from man in many ways likely to be of importance for understanding the different consequences of exposure to chemical carcinogens during fetal versus adult life. Among these are the very much more rapid rates of fetal and neonatal development and maturation in rodents, a factor which may be responsible for the failure so far to observe any tumors strikingly different from those inducible in adults in rodents exposed in utero to potent chemical carcinogens. This project will provide empirical data indicating whether adult or pediatric types of tumors are more readily induced transplacentally in primates.

Proposed Course: This project is an exploratory program to demonstrate transplacental chemical carcinogenesis in a subhuman primate species. Particular emphasis will be placed on defining the periods of maximal sensitivity of different organ systems and demonstrating parallels between both the morphological and clinical behavior of corresponding human tumors, and the results obtained in rodents with the same carcinogen. Comparative pharmacologic studies of the rate and extent of transplacental passage and organ/tissue localization of ENU in rodent species and in patas monkeys will also be made.

Date Contract Initiated: June 28, 1972

Current Annual Level: $105,000

DARTMOUTH COLLEGE (NO1-CP-33378)

Title: Enhanced Delivery of Synthetic Nitroso Compounds to the Pancreas in Rats

Contractor's Project Director: Dr. Daniel S. Longnecker

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: The principal goals of this contract are (1) to asses by in vitro procedures the potential carcinogenicity of selected derivatives of molecules known to localize in the rat pancreas, and (2) to conduct long-term studies in rats with these compounds in an effort to create an animal model of human pancreatic adenocarcinoma.

Major Findings: A series of amino acid analogs and newly synthesized nitroso derivatives of serine, lysine, and neutral red has been screened for mutagenicity in two bacterial systems (pol A⁻ E. coli repair test, and his⁻ Salmonella typhimurium reversion test). Azaserine has shown direct activity in both systems. The following compounds have screened positive with metabolic activation in the E. coli system: O-(N-nitrososarcosyl)-L-serine, O-(N-nitroso-N-methyl-β-alanyl)-L-serine, melphalan, and O-(N-methyl-N-nitroso-5-aminovalyl)-L-serine.

Tissue localization studies with ^{3}H-azaserine have shown that pancreas is second only to kidney in concentration of acid soluble radioactivity after azaserine injection and that it exceeds liver, intestine, spleen, testis, muscle, and thymus. An acid insoluble (macromolecular) fraction from pancreas contained the highest specific activity of any of these tissues following ^{3}H-azaserine injection.

Long-term studies have been initiated with azaserine. Preliminary results from rats autopsied 3 1/2 (1), 6 (16), and 8 (1) months after initiation of treatment have shown hyperplastic nodules in exocrine pancreas in all (18/18) rats and small exocrine adenomas in 3/18 rats. No malignant tumors have been found. One of six control rats had one hyperplastic nodule in exocrine pancreas.

Long-term in vivo study of the carcinogenicity of O-(N-nitroso-N-methyl-β-alanyl)-L-serine is in the first few months.

Significance to Biomedical Research and the Program of the Institute: This approach to the problem of establishing a biological model of pancreatic carcinogenesis is most important to the Carcinogenesis Program. Until such time that a reproducible, experimental protocol is available which characteristically induces cancer of the pancreas, the research necessary to enable an understanding of the cause and ultimate prevention of this disease will be markedly restricted. Model development is one of the major goals, and this project, along with others, is designed specifically to reach this goal.

Proposed Course: Long-term studies of the carcinogenicity of azaserine and O-(N-nitroso-N-methyl-β-alanyl)-L-serine in rats will be continued. Localization studies and preliminary toxicologic studies of additional nitroso derivatives of amino acids are to be carried out preparatory to selection of additional compounds for long-term in vivo studies.

Date Contract Initiated: June 28, 1972

Current Annual Level: $90,770

HAZLETON LABORATORIES, INC. (NO1-CP-23287)

Title: Induction of Adenocarcinoma in Dog Prostate

Contractor's Project Director: Dr. Dan W. Dalgard

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: The primary objective is to develop a protocol, using chemical carcinogens, which results in the development of adenocarcinoma of the canine prostate. Animals from different age groups with different exogenous hormonal stimulation are used in this study. A secondary objective is to establish the normal biologic profile of dogs from birth through puberty and into adulthood. This would include anatomic, histologic, morphologic and histochemical definition of the prostate of various age groups of dogs.

Major Findings: Fibrosarcomas have been induced in nine (39%) sexually immature dogs which received 2-4 injections of 3-methylcholanthrene directly into the prostate. Tumors occurred in six of eleven (54%) dogs castrated prior to inoculation and in three of eight (37%) intact dogs while no tumors were induced in four animals which received supplementary androgens. The animals were approximately four months old when first inoculated and tumors were detected 8 to 12 months later.

Attempts to develop a sustained release devise for continuous administration of carcinogen have been discontinued. Although the desired tumor is an adenocarcinoma, it was felt that the relative ease with which fibrosarcomas were induced negated the need for a sustained delivery system. In addition, it would appear that castration in the sexually immature animals is counterproductive in that it prevents the development of the glandular component of the gland. Consequently, it is being eliminated in the immature groups and its appropriateness in mature animals will be evaluated.

Significance to Biomedical Research and the Program of the Institute: The need for an animal model for the study of prostatic adenocarcinoma is well recognized. The similarities with respect to the incidence, and the clinical and histologic picture of age-associated prostatic conditions in man and dog, certainly make the dog a desirous animal model. If the normal biologic profile can be established for several distinct age groups of dogs it will allow for more meaningful interpretation of the profile of animals with spontaneous and/or induced lesions. It will also permit a more logical experimental approach to induction of tumors.

Proposed Course: The original design which employs three distinct age groups of dogs, three distinct classes of carcinogens, and manipulation of the hormone system will be continued in its implementation. As soon as sufficient numbers of aged animals are obtained, this aspect of the study will be initiated.

Date Contract Initiated: June 24, 1972

Current Annual Level: $118,014

KANSAS, UNIVERSITY OF (NO1-CP-23271)

Title: Histogenesis of Guinea Pig Pancreatic Adenocarcinoma

Contractor's Project Director: Dr. Janardan K. Reddy

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: This project was initiated to confirm earlier reports of induction of adenocarcinoma of the pancreas and stomach in guinea pigs by N-methyl-N-nitrosourea and N-methyl-N-nitrosourethane and to study the histogenesis of the pancreatic tumors. An additional objective of this project is to manipulate this model so as to reduce the induction period and increase the incidence of pancreatic adenocarcinoma.

Major Findings: The use of tritiated methylnitrosourea has permitted the detection of its incorporation into the RNA and DNA of guinea pig pancreas. The incorporation of labeled carcinogen is significantly increased during ethionine induced pancreatic regeneration. Studies are in progress to determine if this increased carcinogen binding reflects high carcinogenic susceptibility of this regenerating pancreas.

Adenocarcinoma of the pancreas developed by 36 weeks in 4 of 11 inbred guinea pigs (strain 13, NIH), that survived once weekly intragastric administration of methylnitrosourea in a dose of 10 mg/kg.b.wt. for over six months. The short induction period suggests that this inbred strain of guinea pigs may be of considerable value in developing a suitable biological model of pancreatic adenocarcinoma.

Significance to Biomedical Research and the Program of the Institute: The incidence of pancreatic adenocarcinoma in this country is progressively increasing, and there is no reliable animal model of this tumor. The development of a biological model of pancreatic carcinoma is most important to the Carcinogenesis Program. Until such time as a reproducible, experimental protocol is available, which characteristically induces cancer of the pancreas, the research necessary to enable an understanding of the cause and prevention of this disease will be markedly restricted. Model development is one of the major goals and this project, along with others, is designed specifically to achieve this goal.

Proposed Course: Additional studies, using large numbers of inbred strain 13 guinea pigs, will be undertaken to confirm the rapid development of pancreatic tumors in this inbred strain. Sequential morphologic studies to determine the histogenesis and progression of this cancer will be expanded. Attempts to increase the incidence of tumors will be made, using various pancreaticotoxic agents such as cobaltous chloride, ethanol, lasiocarpine, or 4-hydroxyaminoquinoline-1-oxide.

Date Contract Initiated: June 10, 1972

Current Annual Level: $109,451

LITTON BIONETICS, INC. (NO1-CP-22063)

Title: Resource for Preparation and Examination of Experimental Biological Material for Histological and Ultrastructural Study

Contractor's Project Director: Dr. Elizabeth W. Kingsbury

Project Officer (NCI): Dr. Curtis Harris

Objectives: The principal objective is the preparation and examination of tissues by light and electron microscopy. The following areas are to be studied: (1) The pathogenesis of chemically induced tumors of various organs such as prostate, pancreas, mammary gland, and lung. (2) The autoradiographic localization of labelled carcinogens, hormones, and other relevant biological compounds such as dimethylbenzanthracene, ketinyl acetate, vitamin A and detection of any ultrastructural evidence of their effects in tissues and cell cultures. A second objective is to provide various light and electron microscopic support services, including autoradiography, for NCI investigators and contractors.

Major Findings: The development of the normal rat prostate in ten different age groups, prenatal to adult, has been studied by light and electron microscopy and the results have been submitted for publication.

Cell cultures of rat mammary gland are being studied by electron microscopy and autoradiography. The effect of insulin, prolactin and hydrocortisone on the proliferation and ultrastructure of these cells is being studied. In addition, cell pellets and mammary tissue from rats of different ages which have received ^{3}H-DMBA have been processed for autoradiography to determine the localization and level of uptake of ^{3}H-DMBA. The susceptibility of the rat mammary gland to the oncogenic effects of DMBA is age dependent. Autoradiographic slides from the above studies have been sent to the NIH for evaluation. These initial projects should establish the basic morphology and cell growth parameters for further studies in rat mammary carcinogenesis.

The following projects have also been completed: (1) The ultrastructural investigation of a possible dog prostate cell line confirmed the epithelial nature of squamous cells but failed to produce any evidence of their prostatic origin. (2) To investigate possible ultrastructural changes due to increased RNA content of mouse epidermal cells, the cells were treated with retinyl acetate (RA) and hormones, each of which increased RNA content. Even after treatment with both RA and hormones, which has a synergistic effect (e.g., increasing RNA to a much greater extent than with RA or hormones alone) no morphological differences, such as nucleolar or cytoplasmic changes, could be detected. (3) Ten autoradiographic projects were submitted which were processed and sent to NIH for further evaluation. Experimental results will be reported elsewhere.

Significance to Biomedical Research and the Program of the Institute: Increasing emphasis has been placed on man's environment in that exposure to chemical carcinogens is on the rise. To decrease the effects of these carcinogens and to develop procedures for protection, their modes of action must be

better understood and defined.

Proposed Course: The morphological description of the rat prostate is to be completed at the ultrastructural level so that future work can begin on studying the effects of hormones and carcinogens on the prostate. Now that cell growth parameters have been established, work will continue to determine why rat mammary tissue becomes highly susceptible to chemical carcinogens at a particular age. Support services will be continued as designated by the project officer.

Date Contract Initiated: December 1, 1971

Current Annual Level: $104,481

MARYLAND, UNIVERSITY OF (NIH-NCI-E-43279)

Title: Studies on the Histogenesis of Renal Carcinoma

Contractor's Project Director: Dr. Benjamin F. Trump

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: The objective of this contract is to develop a model for renal carcinoma in experimental animals. The present model uses a carcinogen, N-4-4' fluorobiphenylacetamide (FBA), administered in the diet to rats. The focus of the study is the definition of the progression of changes in the tubule from normal to malignant lesions and the correlation of each stage with similar lesions occurring in the human kidney. In addition, the biological behavior of such rat lesions are to be determined by assessing transplantability and reversibility upon withdrawal of dietary administration of carcinogen.

Major Findings: Groups of male F344 rats fed a diet containing 0.04% FBA develop large tumors in the kidney cortex in 75% of the animals by approximately one year. The tumors are frequently multiple and vary in size from pinpoint to several centimeters in diameter. The largest tumors observed so far were approximately 1.5 - 2 cm. in diameter and often showed necrosis in the center of the mass. All tumors appeared to originate within the cortex, although in the case of the large tumors they occupied most of the renal mass. Determination of the precise site of origin in the proximal tubule will require further study.

The tumors all appeared to be epithelial, and both solid and papillary patterns were seen; sarcomas have not been identified. The tumor cells were cuboidal to columnar, often showed specializations of the cell membrane resembling the brush border of proximal tubules, and often showed thick basement membrane material both at the cell bases and between the cells in other larger tumor masses. Electron microscopic characteristics resembled those published for the human.

With respect to histogenesis, the earliest hyperplasias were seen by 12 weeks and consisted of tubular forms having small crowded, basophilic cells with small, dense nuclei. Occasional cells with giant nuclei were also seen. By 24 weeks larger hyperplastic nodules often appeared to originate from growth of cells into the lumens of tubules. Again, these cells had a well-developed brush border and a terminal web-like area just beneath the plasma membrane. By 36 weeks, multiple nodules of hyperplasia were identified. These nodules became larger and began to resemble the cells in the large tumors seen by one year. Transplantation studies are underway and indicate that even very small lesions (less than 2 mm in diameter) can be transplanted in one generation so far.

Histochemically, the tumors showed a marked depression of glucose-6-phosphatase activity and succinate dehydrogenase activity, while the activities of glucose-6-phosphate dehydrogenase, lactate dehydrogenase, and alpha-glycerol-phosphate dehydrogenase were similarly enhanced compared to the strongest reacting components of the normal nephron.

Studies of human renal carcinoma have revealed the common presence of multiple small lesions similar to those seen in the rat, ranging from focal changes involving a few cells in a proximal tubule to small areas of hyperplasia. So far the human lesions resemble those seen in the rat model. Electron microscopic studies to date on the human also show morphology compatible with that seen in the rat, though many further studies are presently underway.

Significance to Biomedical Research and the Program of the Institute: These studies are mainly of significance in that they appear promising with respect to development of an animal model of human renal carcinoma and to development of histogenetic stages of human carcinoma development. This would obviously be of great value in terms of studying carcinogenesis of renal carcinoma and the environmental or other factors that influence the course of this malignancy.

Proposed Course: Future efforts will be concentrated on performing similar histogenesis studies of human renal carcinomas. Human observations will be compared to those of the rat model in an attempt to draw appropriate conclusions about the usefulness of the animal to study of the mechanism of human neoplastic proliferation.

Date Contract Initiated: February 1, 1972

Current Annual Level: $99,925

MASON RESEARCH INSTITUTE (NO1-CP-23238)

Title: Uptake and Excretion of Carcinogens in and Their Effects on the Prostate

Contractor's Project Director: Dr. Emil R. Smith

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: The principal goal of this contract is to measure the levels of selected carcinogens in the blood, the prostate gland, and the prostatic fluid of rats and dogs after administration of such substances by an appropriate route. Those agents having the highest affinity for the prostate will then be employed in chronic studies in rats and dogs in an attempt to produce adenocarcinoma of the prostate.

Major Findings: Acute experiments were conducted in both rats and dogs with cadmium (Cd), 7,12-dimethylbenzanthracene (DMBA), 3-methylcholanthrene (MCA), and 2-acetylaminofluorene (2-AAF). While all four of these substances penetrated the prostate after systemic administration and could be found in prostatic fluid, none was highly concentrated by the gland. Accordingly, emphasis was shifted to the development of new methods of treatment. Subsequent experiments in rats revealed that when solutions of Cd were injected into the vas deferens in the direction of the prostate-bladder complex, they resulted in the retrograde penetration of Cd into the prostate and the development of microscopic changes within the gland. Similar injections of DMBA produced microscopic alterations, indicating that it also penetrated the gland. In dogs with prostatic fistulas, it was found that flusing of the fistula with solution of Cd led to a retrograde passage of Cd into the prostate and that repeated flusings led, with time, to the development of microscopic changes in the gland. Since the penetration of these substances into the prostate following their deposition into the urethra must result from passage through the secretory epithelium, these new techniques offer new approaches to the production of adenocarcinoma of the prostate.

Significance to Biomedical Research and the Program of the Institute: Although prostatic adenocarcinoma is a major cause of human cancer deaths, there are no experimental models for this kind of cancer and very little work is being done to develop such models. This project is one of several developed by the Biological Models Segment in order to stimulate research on the etiology and pathogenesis of adenocarcinoma of the prostate. This specific project is designed to aid in the selection of the appropriate chemical carcinogen, species, and treatment procedure (route, dose, duration, etc.) for attempts to produce an animal model of prostatic adenocarcinoma.

Proposed Course: Subacute and chronic experiments have been, or are now being, started in which solutions of known carcinogens are injected into the vas deferens of rats or administered in a retrograde manner directly into the prostatic fistulas in dogs. These experiments will require one to two years for completion.

Date Contract Initiated: June 15, 1972

Current Annual Level: $120,523

MICHIGAN, UNIVERSITY OF (NO1-CP-33303)

Title: Isolation and Purification of Epidermal Chalone

Contractor's Project Director: Dr. John J. Voorhees

Project Officer (NCI): Dr. Henry Hennings

Objectives: Chalones have been defined as tissue-specific, species-non-specific inhibitors of cell proliferation. The primary objective of this contract is the isolation and purification of an epidermal chalone. The inhibitor which acts at the G_1 phase of the cell cycle will be purified and characterized.

Major Findings: Epidermal cells and fibroblasts from newborn Balb/c mice are growing in cell culture. The epidermal cells have been characterized by electron microscopy (presence of tonofilaments and desmosomes), histochemical enzymatic assay (negative for leucine aminopeptidase), and light microscopy (flat cuboidal cells growing from clusters). In contrast, the fibroblasts contain rough endoplasmic reticulum, leucine aminopeptidase activity, and spindle-shaped cells that grow from single cell starting points. Newborn rat epidermis is used for the isolation of the epidermal chalone. The particulate fraction obtained by 600g centrifugation of the homogenate obtained with a Polytron was treated with sodium dodecyl sulfate. After centrifugation at 104,000g, the supernatant fractions contained chalone activity and responded in a dose dependent manner in the in vivo assay. This fraction was further purified on a Bio-Gel P-30 column. The fraction excluded from the column contained chalone activity as determined by the in vitro assay on epidermal cells and on fibroblasts.

Significance to Biomedical Research and the Program of the Institute: Since the control of proliferation is of great importance in controlling malignant growth, studies of systems involving the inhibition of cell proliferation in a tissue will probably produce information applicable to the general control of proliferation. Once the chalone is purified, the mechanism by which it exerts its inhibitory activity might indicate what malfunction occurs in malignant proliferation.

Proposed Course: Information available in the literature indicates a strong correlation between events at the cell surface and altered rates of cell proliferation. The initial purification procedure for the epidermal chalone points to the cell surface or cell membrane as the source of the chalone activity. Therefore, the cell membrane-cell surface material will be isolated from epidermal cells from newborn rats that have regenerated their cell surface material during a 24 hour incubation period in tissue culture medium containing ^{3}H-glucosamine, ^{3}H-fucose, and ^{3}H-galactose. This enrichment of starting material for chalone activity should facilitate its purification. The chalone activity would then be obtained by trypsinization of cell surface material or by solubilizing the cell membrane. Purification of the material on Bio-Gel columns, DEAE cellulose columns, and ultrafiltration should yield a very active chalone fraction. All of the fractions will be tested in the in vitro tissue culture assay system. The in vivo assay will be employed to confirm these results.

Date Contract Initiated: April 1, 1972

Current Annual Level: $112,793

MICHIGAN, UNIVERSITY OF (NO1-CP-33331)

Title: Organ Culture of the Prostate: A Biological Model

Contractor's Project Director: Dr. Raymond H. Kahn

Project Officer (NCI): Dr. Stuart Yuspa

Objectives: (1) To ascertain the physical and chemical requirements for the long-term maintenance of the normal canine and human adult prostate in vitro in a histotypically normal manner, giving particular attention to the glandular epithelium; and (2) to establish the structural and histochemical criteria for the normal canine and human prostate, both in vivo and in vitro.

Major Findings: A modified organ culture technique has been developed which perfuses nutrient media through the culture. Emphasis is being placed on some of the environmental factors necessary to maintain the prostate in vitro. Conditions currently under investigation include the gaseous environment and hormonal additives; namely, insulin and testosterone. To date, viable explants of the canine and human prostate have been cultivated for periods of up to 15 days. While the glandular epithelium is maintained in a viable state, it is markedly changed from that seen in vivo. Preliminary studies on the histochemical markers which correlate viability in vitro with the prostate in vivo have been initiated.

Significance to Biomedical Research and the Program of the Institute: While organ culture can provide a reasonable biological model to the prostate in vivo, unfortunately, the nutritive and system-dependent factors (e.g., pH, osmolatity, gaseous) necessary for the long-term maintenance of this tissue have not been adequately investigated to date. More importantly, most experimental studies with the prostate, using organ culture, have been of the rat and mouse prostate. Since only the dog and human prostate undergo spontaneous neoplasia in vivo, an understanding of the etiology of prostatic adenocarcinoma in these two species seems warranted.

Proposed Course: The effect of nutritional (e.g., glucose, hormones) and system-dependent factors (e.g., pH, osmolality, oxygen tension) in an attempt to foster the retention of histotypical characteristics of the prostatic parenchyma will be studied. The results of these studies in vitro will be compared structurally (light and electron microscopy) and functionally (histochemically) with comparable lobes in vivo. Attempts to establish optima for pH and oxygen, and selecting a single medium for subsequent studies will be initially performed. Moreover, influence of glucose and osmolality will be studied.

Date Contract Initiated: June 25, 1973

Current Annual Level: $228,071

MICROBIOLOGICAL ASSOCIATES INC. (NO1-CP-02199)

Title: Laboratory Service for Support in Carcinogenesis Bioassay and Related Activities

Contractor's Project Director: Dr. Russell Madison

Project Officer (NCI): Dr. Jerry Rice

Objectives: This contract provides long-term animal holding facilities and associated histopathology, biochemistry, and cell culture laboratories for studies on animal models of major forms of human cancer. These are carried out on a collaborative basis between the contractor's personnel, NCI investigators, and other contractors within the Biological Models Segment, and emphasize in vivo carcinogenesis experiments on carcinoma of the lung, pancreas, gastrointestinal tract, prostate and mammary gland, on the various forms of pediatric solid tumors, and on the role of transplacental exposure to carcinogens in the development of both adult and pediatric neoplasms.

Major Findings: Direct administration of 1-ethyl-1-nitrosourea to the rat prostate has resulted in the development of prostatic adenocarcinoma, proving that this form of cancer is chemically inducible in this species. Aromatic hydrocarbon carcinogens used in this way give rise to soft-tissue sarcomas and/or squamous-cell carcinomas, rather than adenocarcinomas. Studies on pituitary- and androgen-mediated stimulation of the prostate as co-carcinogenic factors are in progress. Studies of ethionine-mediated pancreatic necrosis and regeneration in combination with oral nitrosamide carcinogens in Hartley guinea pigs have yielded hyperplastic acinar and ductal lesions but no tumors. These studies are being extended to the strain 13 guinea pig, in collaboration with contractors at the University of Kansas who have shown that this strain is susceptible to pancreatic adenocarcinoma induction by nitrosamides. Studies are in progress on the (1) co-carcinogenic effects of various environmental contaminants and (2) inhibitory effects of vitamin A and of non-specific immunoprophylaxis on induction of bronchogenic squamous-cell carcinoma in the Syrian hamster, together with dose-response studies on the development of this neoplasm from exposure to nitrosamide carcinogens. Transplacental carcinogenesis studies with aromatic hydrocarbon carcinogens in rats have yielded both neurogenic and nephroblastic tumors, confirming the potential of this species for model studies on Wilms' tumor and the high susceptibility of the rat nervous system to a wide spectrum of chemical carcinogens. Studies have begun on the comparative carcinogenic effect of dimethylnitrosamine (DMN) and synthetic derivatives of its assumed active metabolite in adult, newborn, and fetal rats, to define the role of α-hydroxylation in the metabolism of dialkylnitrosamines and to evaluate the relative importance of (1) maternal vs. fetal/placental metabolism and (2) transplacental passage of precursor and metabolites in the relative resistance of the rodent fetus to transplacental

carcinogenesis by DMN and its homologs.

Significance to Biomedical Research and the Program of the Institute: The commitment by both NCI and contract-supported laboratories within the Biological Models Segment to the development and etiologic study of animal models of the major neoplastic diseases of man requires a facility for long-term animal studies to be used for this research. This contract provides such a resource and makes possible rapid implementation of new ideas in this area, as well as collaboration among several laboratories with complementary expertise.

Proposed Course: A major proportion of this year's budget consisted of equipment and furniture expenses for enlargement of this facility. Additional space thus prepared will be available during the next fiscal year, will enable expansion of current efforts on lung, pancreatic, and pediatric neoplasms to include *in vitro* approaches, and will allow initiation of *in vivo* programs in other areas, particularly gastrointestinal cancer.

The pancreatic carcinoma studies will be extended to strain 13 guinea pigs, which are known to respond more readily to nitrosamide carcinogens. Studies on the modulating role of vitamin A in the development of bronchogenic squamous cell carcinoma will be extended to other organ systems which are technically easier to manipulate but which provide experimental systems for analysis of factors affecting the development of squamous cell neoplasms. These include mouse epidermis and rat urinary bladder. Tissue and organ culture studies will be carried out in parallel with the *in vivo* experiments for controlled morphological and biochemical characterization of cells responding to the vitamin during oncogenesis, utilizing for the first time the expanded laboratory facilities developed during the current contract year.

Date Contract Initiated: June 4, 1970

Current Annual Level: $390,000

NEBRASKA, UNIVERSITY OF (at Lincoln (NO1-CP-33289)

Title: Chemical Carcinogen-Induced Noduligenesis and Tumorigenesis in Whole Mouse Mammary Gland Organ Culture

Contractor's Project Director: Dr. Mihir Banerjee

Project Officer (NCI): Dr. Richard R. Bates

Objectives: To determine whether preneoplastic (nodule) or neoplastic "transformation" of mouse mammary epithelium can be induced by chemical carcinogens during hormone induced development of the mammary gland in organ culture.

Major Findings: The results of the following initial studies were reported previously: (1) The second thoracic mammary gland of the 4-5 week old female mouse is most suitable for whole mammary gland organ culture. (2) The full

lobulo-alveolar growth induced in the gland by growth-promoting hormones during the initial 5-7 days incubation can be maintained up to three weeks in organ culture containing the same hormones. (3) But incubation of the fully developed gland in medium with insulin alone results in regression of the alveolar structures, and the parenchyma resembles the involuted mammary gland of the weaned mother. This phenomenon of hormone regulated growth and regression of the alveolar structures in whole mammary gland organ culture seems to simulate the endocrine regulated developmental cycle of the mammary tissue in the animal. The knowledge obtained from the preceding observations was utilized in designing the following experiments pertaining to chemical-carcinogen-induced "transformation" of mammary cells in organ culture. Results of some of these experiments can now be reported.

The entire second thoracic mammary gland of young C3H/f BALB/c or BALB/c female mice was induced to full lobulo-alveolar growth within five days in organ culture supplemented with the growth-promoting hormones. The rapidly proliferative mammary cells were treated with 1 µg/ml, 7,12-dimethylbenz-anthracene (DMBA) or 5 µg/ml, 3-methylcholanthrene (MCA)during the initial 24-72 h in culture, the medium was changed and incubation was continued in the growth-promoting medium without carcinogens for another 3-5 days. The full-grown parenchyma was then incubated in a hormone deficient medium containing only insulin (regression medium). The glands in the parallel control experiments were also incubated in the similar manner but no carcinogen treatment was given. As expected, after incubation in the regression medium, most alveolar structures were regressed, but numerous isolated clusters of alveolar structures were distinctly visible in a high frequency of the carcinogen-treated glands. The non-regressed alveolar nodule-like lesions present in the carcinogen-treated glands morphologically resembled the well-established precancerous nodules of the mouse mammary gland in vivo.

The maintenance of the alveolar structures in a hormone deficient medium strongly suggests an altered hormonal requirement of these alveoli in the nodule-like lesions induced in organ culture. A similar altered hormonal requirement also characterizes the precancerous mammary nodules in vivo. Histologically, the alveolar nodule-like structures induced in organ culture were virtually indistinguishable from the precancerous, hyperplastic mammary nodules, induced in the animal by viruses, chemical carcinogens, or hormones. This finding thus provides the first demonstration of nodular "transformation" of mammary epithelium by chemical carcinogens in organ culture.

Significance to Biomedical Research and the Program of the Institute: The Biological Models Segment has the goal of developing appropriate animal models for studying the biology and pathogenesis of major forms of human cancer. Cell and organ culture systems provide an effective tool for these studies without involving the complexities of the entire animal. The unique organ culture procedure utilized in this project permits hormonal induction of development of the entire breast in the culture medium. The results presented above so far strongly indicate that hyperplastic alveolar nodules (similar to the preneoplastic mammary nodules in vivo) can be induced by chemical carcinogens in the mammary gland within 2-3 weeks in organ culture (in animal, the same process may take six months to a year). These observations are

considered very encouraging. Consistent with the objectives of the Biological Models Segment, development of an in vitro carcinogenesis system in organ culture of whole mammary gland will provide an important tool for studying the pathology of hormone-carcinogen interactions in breast cancer and also a rapid relatively less expensive assay system for testing various environmental carcinogens exclusively in the Petri dish.

Proposed Course: The reproducibility of the chemical carcinogen induction of hyperplastic alveolar nodules in the whole mammary gland organ culture so far seems good, but more experiments will be needed. Various madifications of the procedure will be used to increase the incidence of the nodule-like lesions in the treated glands. The crucial part of the study will be to determine the precancerous nature of the nodule-like lesions induced by the carcinogens in the mammary gland in culture. This will be tested by transplantation of the nodule-like tissues into the animal. Two types of transplantation procedure will be used: (1) transplantation of tissue samples containing the nodule-like lesions into gland-free mammary fat-pad of young females, and (2) transplantation of the entire mammary gland containing the nodule-like lesion induced in vitro onto the peritoneal wall of the animal. Production of mammary adenocarcinomas by the explants will be indicative of the preneoplastic nature of the lesions. Attempts will be made to develop an improved method of detecting the nodule-like lesions in unstained glands, and this will provide more precise tissue sample for transplantation.

Date Contract Initiated: April 1, 1972

Current Annual Level: $108,000

ORGANIZATION FOR HEALTH RESEARCH (TNO) RADIOBIOLOGICAL INSTITUTE (NO1-CP-33330)

Title: Synergistic Interaction of Hormones and Neutrons Radiation on Mammary Gland Carcinogenesis

Contractor's Project Director: Dr. Johannes J. Broerse

Project Officers (NCI): Dr. Norbert P. Page
Dr. D. Jane Taylor
Dr. Douglas Janss

Objectives: To evaluate synergistic interactions between neutron radiation and estrogenic hormones on the induction of mammary gland carcinogenesis. These studies involve a comparison of three different rat strains, one of low susceptibility (BN), one of medium (WAG), and one of high susceptibility (Sprague Dawley) to tumor induction. In addition, these studies will evaluate the effect of different neutron energies (range .5 Mev - 15 Mev) and three dose levels of 250 Kvp x-ray.

Major Findings: No findings can be reported at this time, as these studies have just been initiated and are of a long-term nature. However, the experimental groups are in the process of being set up, equipment and animal resources have been obtained, and the studies are now underway.

Significance to Biomedical Research and the Program of the Institute: Previous work conducted at the Alton Oschner Foundation indicates that x-radiation and diethylstilbesterol interact in a synergistic manner in inducing mammary tumors in female A x C rats. Studies conducted at other laboratories indicate that neutron radiation is considerably more effective in inducing mammary neoplasia than is x-ray. In medical practice many females are exposed to combinations of radiation and estrogenic hormones. In addition, a significant exposure to neutron radiation may occur in industry, research, and medicine. There is evidence that exposure of women to fluoroscopic examinations, nuclear radiation, and x-rays for post-partum mastitis increases the risk of developing breast cancer. With the more frequent use of neutron radiation, along with increased utilization of sex steroids for contraceptive and replacement therapy, interactions of neutron radiation and sex steroids should be evaluated as has been done with x-radiation and hormones. This project should provide information to assess the neutron/hormone relationships as well as provide information on differences in population sensitivities.

Proposed Course: The initial studies will involve the exposure of the WAG rats which have intermediate susceptibility to mammary tumor induction. The low and high susceptible strains will be entered into the experiments during the second year.

Date Contract Initiated: June 25, 1973

Current Annual Level: $195,201

PAPANICOLAOU CANCER RESEARCH INSTITUTE (NO1-CP-23288)

Title: Induction of Prostatic Adenocarcinoma in the Rat

Contractor's Project Director: Dr. Wilhelmina F. Dunning

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: To attempt induction of prostatic adenocarcinoma in an inbred A x C line 9935 Irish male and Copenhagen line 2331 male rats by the indirect application of 3-methylcholanthrene or 7,12-dimethylbenzanthracene combined with androgenic stimulation of the prostatic epithelium.

Major Findings: Currently 14 groups of A x C or Copenhagen males are under observation after a maximum of eight months of continuous androgenic stimulation and the ingestion of 20 to 50 mg of 3-methylcholanthrene or 7,12-dimethylbenzanthracene. The maximum period of observation for the initially treated groups is 573 days. No gross tumors of the prostate have been observed.

Among the rats that received 7,12-dimethylbenzanthracene, 50 have come to necropsy. All have shown cystic or adenomatous hyperplasia of the prostate and 25 or 50 percent have died with induced malignant disease in other locations. In contrast, only 31 of the similarly treated rats that received

3-methylcholanthrene have died and none with induced gross neoplasms.

Significance to Biomedical Research and the Program of the Institute: Establishing a biological model of prostatic carcinogenesis is most important to the Carcinogenesis Program. Until such time that a reproducible, experimental protocol is available which characteristically induces adenocarcinoma of the prostate, the research necessary to enable an understanding of the cause and ultimate prevention of this disease will be markedly restricted.

Proposed Course: Animals will continue to be periodically observed for morphological alterations in the prostate and other major glands in an attempt to discern the influence of hormones on the carcinogenic process and derive insight into a suitable protocol for induction of prostatic adenocarcinoma.

Date Contract Initiated: June 16, 1972

Current Annual Level: $24,578

PORTLAND STATE UNIVERSITY (NO1-CP-43256)

Title: Isolation and Maintenance of Normal Human Prostatic Epithelial Cell Lines

Contractor's Project Director: Dr. Thelma N. Fisher

Project Officer (NCI): Dr. Stuart H. Yuspa

Objectives: The purpose of this project is to isolate and propagate normal human prostatic epithelium so that such tissue preparations are available for numerous studies involving mode of action of carcinogenic substances. To achieve this goal, it is essential that proof of the epithelial nature of these isolates be established by various approaches such as observing typical epithelial morphology, by providing three-dimensional growth on matrices in vitro, by assessing the secretory nature of these tissues, and by measuring response to testosterone stimulation. The elimination of fibroblasts from mixed cultures is a significant goal. Frequent surveys for normal karyotype patterns are performed in determining whether or not spontaneous malignant conversions occur. If human tissue culture models can be maintained for numerous transfers and stored successfully, the models will be useful to the scientific community in studying the cause and prevention of cancer.

Major Findings: Explants of prostatic tissues removed surgically from 75 human subjects were cultivated. These individuals ranged in age from 18 to 94 years. Two patients were in the 30-39 year age group, one patient was 43, eight patients were in the 50 to 59 year age group, while the remainder were 60 years or older. The best epithelial outgrowth achieved to date was that observed for explant cultures of an 18-year-old young man. None of the tissues processed were diagnosed as malignant. Of the 75 patients studied, prostatic tissue from all but two patients provided some sort of cellular outgrowth when explants were cultivated in Eagle's medium containing 20%

fetal bovine serum. Minute amounts of fibroblast extract, putrescine, or testosterone were added to culture fluids in certain experiments. Four patients provided tissues which, by microscopic examinations, gave 100% epithelial outgrowth; heavy fibroblast outgrowth was obtained from two patients. Tissues of 22 individuals showed epithelial outgrowth predominating, while all other cultures consisted of epithelial-fibroblast cell type mixtures. Probably two fibroblast cell lines have been established permanently since they have been transferred 19 times. No permanent epithelial cell lines have been established to date; however, transfer experiments are underway.

A target cell selection procedure carried out in the presence of μg amounts of testosterone has been designed. Epithelial target cells respond by detaching from plastic surfaces, while fibroblasts remain in position. It is possible to remove single epithelial cells or clones by this method. Anti-fibroblast immunoglobulin, to be used to remove fibroblast contamination in cultures of prostatic explant outgrowth, is in preparation. A pilot autoradiographic experiment designed to label prostatic epithelium receptor sites with ^{3}H testosterone is in progress.

Significance to Biomedical Research and the Program of the Insitute: In order to determine initial reactions involved in the malignant conversion of prostatic epithelium, the development of a normal prostatic human epithelial tissue culture cell line is desirable and essential. This is an important goal of the Biological Models Segment.

Proposed Course: An important thrust for the present is to attempt to obtain human tissue from younger human subjects such as that from accident cases, neonatal deaths, and stillborns. Although fibroblast transfer and growth on artificial matrices has been achieved, this remains a problem for epithelium. The use of various plastic grids, gel foam, and chorioallantoic membranes of fertile eggs will be tested as supports for 3-dimensional growth of first transfer epithelial cell sheets. Several techniques involving trypsin and its inhibitors are planned to remove cell outgrowth from plastic surfaces. In addition to selection and exclusion techniques mentioned, inhibition of fibroblast contamination by antifibroblast antibody is anticipated. Autoradiographic techniques are proposed to show that epithelial growth is target tissue and contains testosterone receptors. Other steroid hormones must be tested as well. A continued search for growth-enhancing factors and optimum nutritional conditions is under investigation. Adequate storage procedures for initial explant material and subsequent epithelial tissue cultures are needed and will be attempted.

Date Contract Initiated: December 28, 1973

Current Annual Level: $48,405

ST. LOUIS UNIVERSITY (NO1-CP-33370)

Title: Synthetic Nitroso Derivatives as a Means for Concentrating Carcinogens in the Pancreas

Contractor's Project Director: Dr. Thomas Curphey

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: The principal objective of this contract is the synthesis of N-nitroso derivatives of specific compounds known or suspected to have a high affinity for the pancreas. Certain derivatives will be labeled with tritium or carbon-14 for tracer analysis.

Major Findings: Efforts have centered on the synthesis of potential carcinogens derived from alpha amino acids. Two types of such carcinogens have been prepared, one a series of serine esters with the general formula $CH_3NNO(CH_2)_n CO\text{-}OCH_2CHNH_2CO_2H$, and the other a series of epsilon lysine amides of general formula $CH_3NNO(CH_2)_nCONH(CH_2)_4CHNH_2CO_2H$. The serine esters with n = 2-5 were prepared by acylation of carbobenzoxyserine benzyl ester with the symmetrical anhydride formed from the appropriate nitrosamino acid and dicyclohexylcarbodiimide, followed by unblocking of the benxyl protecting groups. The lysine amides with n = 2-5 were prepared by acylation of copper (II) lysinate with a mixed anhydride of the appropriate nitrosamino acid, followed by removal of copper with hydrogen sulfide. For the homologue with n = 1, the p-nitrophenyl ester of N-nitrososarcosine was used as acylating agent.

Several of these nitroso-containing amino acids, which have been or are now undergoing biological evaluation under a separate contract, have shown evidence of potential carcinogenicity.

Significance to Biomedical Research and the Program of the Institute: Induction of pancreatic adenocarcinoma in animals is a prime goal of the Biological Models Segment. Such a successful protocol leading to reproducible tumor formation would permit the study of the factors responsible for this malignancy, the early cellular alterations during carcinogenesis, and insight into ways to modify the induction process. The approach of using chemicals that are carcinogenic and preferentially bind or concentrate in the pancreas is not currently feasible. Modification of chemicals known to concentrate in this organ with functional groups known to be carcinogenic may accomplish both tasks simultaneously.

Proposed Course: Large scale preparation of amino acid carcinogens will be undertaken, as required, to support long-term feeding studies conducted under a separate contract. Radiochemical labeling for tracer analysis will be carried out. Additional potential carcinogens will be prepared and submitted for evaluation.

Date Contract Initiated: June 28, 1972

Current Annual Level: $57,800

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NO1-CP-33379)

Title: Gonadal Hormone Effects on the Prostate

Contractor's Project Director: Dr. Sydney A. Shain

Project Officer (NCI): Dr. Douglas H. Janss

Objectives: The primary objective of this contract is to define the comparative endocrinology of the various lobes of the rat and canine prostate in order to provide guidelines for future studies with human tissue and to attempt to define the role of aging in the potentiation of the prostate for tumor development. These studies are divided into two complementary areas of endeavor and include interspecific, interlobular, and interage comparative studies of (1) testosterone metabolism in the rat and dog prostate and the effects of estrogens and antiandrogens upon the testosterone metabolic potential of the rat and dog prostate, and (2) high affinity binding of testosterone, testosterone metabolites, estrogens, and antiandrogens by cytoplasmic receptors of the rat and dog prostate and the subsequent transport and retention of bound steroids by prostatic nuclei.

Major Findings: Comparative studies of testosterone metabolism in the ventral and dorsolateral complex of the rat prostate have demonstrated that the intracellular concentration of testosterone and testosterone metabolites is primarily regulated by reductive metabolism and that the reductive metabolic potential of the dorsolateral prostate is very much greater than that of the ventral prostate. Moreover, the array of testosterone metabolites elaborated by incubations employing both ventral and dorsolateral prostate is dependent upon the concentration of testosterone in the incubation medium. In both tissues, increasing the concentration of testosterone causes an increased production of oxidized metabolites. Aging of the rat results in a dimunition of 30 to 35 percent in the testosterone metabolic capacity and a diminished testosterone reductive metabolic potential in the rat ventral prostate which is manifest as an increased production of oxidized metabolites of testosterone as well as an absolute decrease in the amount of reduced metabolites of testosterone elaborated. The effects of in vitro exposure of ventral and dorsolateral prostate to estradiol upon testosterone metabolism were minimal and variable. Estradiol caused a modest reduction of about 20 percent (ventral prostate) or 5 to 45 percent (dorsolateral prostate) in the total testosterone metabolic capability of prostate from rats 22 to 36 months of age and was without effect in incubations employing tissue from young immature rats. Estradiol had no effect upon the profile of elaborated metabolites in incubations employing ventral prostate; whereas, the effect in dorsolateral tissue incubations ranged from a 105 percent increase to a 50 percent decrease in the value of the ratio of the 17-hydroxy to 17-keto metabolites.

In vivo and in vitro evaluations of ventral prostate cytoplasmic receptor have revealed an aging-associated reduction in receptor content per cell. In Sprague-Dawley animals, a dimunition of slightly greater than 50 percent was first detectable in tissue from animals 8.5 to 10 months of age. In A x C rats, a consistent reduction of slightly greater than 50 percent was detectable in tissue from animals 10.5 months of age, and the reduction was maintained in tissue from animals 18.5 months of age. Receptor was essentially undetectable in tissue from animals 29 to 32 months of age or was present at less than 15 percent of the value seen in young mature A x C rats. The loss of receptor

was demonstrated not to be attributable to an aging-associated production of a unique cytoplasmic factor capable of inactivating the ventral prostate receptor-ligand complex. Dorsolateral cytoplasmic receptor was not demonstrable for either in vivo or in vitro conditions which readily yielded ventral prostate receptor in simultaneous determinations. Dorsolateral prostate possessed a nuclear androgen receptor which was indistinguishable from the ventral prostate nuclear receptor by the criteria of sucrose gradient centrifugation. Nuclear accumulation of 5α-dihydrotestosterone by minces of dorsolateral prostate was a time-dependent process which required 20 to 30 minutes incubation at 37° to achieve apparent saturation. Saturation analysis of dorsolateral nuclear binding sites revealed that this tissue contained about 3400 nuclear 5α-dihydrotestosterone binding sites per cell.

Significance to Biomedical Research and the Program of the Institute: A major goal of the National Cancer Institute is to identify these factors resulting in the development of cancer so as to meaningfully reduce the incidence of the disease. The relationship between aging and the increased incidence of adenocarcinoma of the prostate is well established. This project is a part of a program that will attempt to identify these aging associated changes in prostatic endocrinology which may be causative for the potentiation of the prostate for the development of adenocarcinoma. Identification of causative factors will allow for meaningful endocrinologic manipulation in an attempt to reduce the incidence and morbidity of adenocarcinoma of the prostate.

Proposed Course: This project will continue (a) to define the changing endocrinology of the various lobes of the prostate of the aging rat with respect to both testosterone metabolism and the effects of estrogens and antiandrogens upon testosterone metabolism and to define the mechanism of androgen retention and action in the prostate of the aging rat, and (b) to develop a similar series of evaluations of the endocrinology of the various lobes of the prostate of the aging canine.

Date Contract Initiated: June 28, 1972

Current Annual Level: $105,000

TEMPLE UNIVERSITY (NO1-CP-33262)

Title: Biochemical and Morphologic Components of Hepatic Carcinogenesis

Contractor's Project Director: Dr. Emmanuel Farber

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: To develop a bioassay technique for rapid screening of potential hepatocarcinogens using the hyperplastic nodule as the end-point; to establish the relationship of nodular hyperplasia to the origin of hepatocellular neoplasms; to characterize biochemically and morphologically subcellular markers of the hyperplastic nodule and to determine their occurrence in nodules produced with diverse carcinogens; and to obtain tissue in larger amounts for

these studies. The test system should thus provide a means for rapid identification of hepatic carcinogens and offer insight into molecular mechanisms of pathogenesis.

Known or suspected hepato-carcinogens and hepato-toxins are fed to Wistar or Fischer strain rats in a basal diet of known composition and the production of hyperplastic nodules and/or malignant hepatomas are noted. Relationships of nodular proliferation to tumorigenesis are studied by biochemical, metabolic, and histological observations at the light and electron microscope levels.

Major Findings: A new protein antigen (PN antigen) has been found to be a highly specific marker for hepatocytes in hyperplastic nodules in liver during hepatocarcinogenesis induced by 2-acetylaminofluorene (2-AAF) or ethionine. This new protein marker has been found in every primary hepatoma induced by 2-AAF, ethionine, 3'-methyl-dimethylaminoazobenzene, dimethylnitrosamine or aflatoxin B_1 but is present in only some transplantable Morris hepatomas. It has so far not been found in any normal organ, including liver, serum, fetal liver, fetal serum or amniotic fluid. It has been localizad by immunofluorescence to the cytoplasm of hepatocytes in every hyperplastic nodule, both early and late, and in every primary hepatoma. PN antigen appears in some livers at one and two weeks after initiating an hepatic carcinogen regimen and in every liver after two weeks. The quantitative assay of PN antigen, which is now being developed, gives great promise of offering a quantitative method to identify and measure the number of new preneoplastic cells in the liver. This should allow an accurate, very rapid bioassay for potential carcinogens using an end-point other than initiation and new more quantitative approaches in the study of mechanisms of carcinogenesis.

Significance to Biomedical Research and the Program of the Institute: These new findings have importance in the further understanding of mechanisms of cancer induction by chemicals, and offer a real possibility to distinguish preneoplastic liver cirrhosis in humans from the many cases of cirrhosis not related to liver cancer. In addition, it suggests that preneoplastic-specific cell components may occur in other organs and tissues undergoing neoplastic transformation. This in turn offers the possibility of the development of a much more specific diagnostic test for preneoplastic changes in various organs. With respect to the liver, it offers the most convincing evidence for the preneoplastic role of hyperplastic nodules in the induction of hepatocellular carcinoma.

Proposed Course: (a) To pursue in increasing depth the nature and significance of the new specific marker for preneoplastic liver tissue in mechanisms of carcinogenesis and in the analysis of human liver cancer and liver cirrhosis; (b) to begin to explore the possible presence of analogous markers in preneoplastic cells in other organs or tissues; and (c) to develop a better scientific basis for the rapid bioassay for carcinogenesis, using end-points other than the initial interaction with target macromolecules.

Date Contract Initiated: January 20, 1964

Current Annual Level: $44,063

TENNESSEE, UNIVERSITY OF (NO1-CP-33369)

Title: Maintenance of Organ Explants from Rodent Pancreas

Contractor's Project Director: Dr. Leonard Murrell

Project Officer (NCI): Dr. Stuart Yuspa

Objectives: The technique of organ culture is being used increasingly to bridge the gap between cell culture and in vivo conditions. The ability to maintain a differentiated tissue in culture permits direct histological and/or biochemical assessment of the effect(s) of chemical carcinogens on the tissue itself, and, simultaneously, evaluation of products released by cultured explants. It is becoming apparent that each organ has specific culture requirements. In addition to the requirements common to all in vitro environments (temperature, humidity, etc.) it is now recognized that many cultured organs require rigorous control (at specific levels) of pH, osmolality, hormonal milieu, and other system dependent factors, such as oxygen concentration. Therefore, any realistic approach to maintaining pancreas in culture must include detailed assessment of each of these conditions, as well as evaluation of their possible interactions. The present project attempts to elucidate some of the factors necessary to maintain rodent pancreas--and ultimately human pancreas--in organ culture. After development of appropriate culture conditions, the organ cultures will be used in studies of chemical carcinogenesis of the pancreas.

Major Findings: Early effort in this program confirmed that classic organ culture methods were not suitable for pancreatic acinar tissue. Therefore, a circumfusion culture system capable of exchanging culture medium, in whole or in part, at predetermined intervals was designed. Survival of cultures is monitored by evaluation of amylase released into culture medium (or contained in cultured tissues), and by examination of histological preparations from cultured tissues. Major work thus far has employed a serum-free, chemically-defined culture medium. Circumfusion cultures of explants prepared from neonatal rat pancreas have established that maintenance of viable acinar tissue is dependent in part on (1) stabilization of culture medium pH at 7.5$\pm$ 0.1, (2) high levels of oxygen in the gas phase, and (3) rate of medium circumfusion. Explant size appears to be non-critical, provided one dimension of each explant is no more than 1.0 mm. The level of gas phase oxygen appears to interact with the rate of medium circumfusion, to produce best survival with high oxygen levels, and exchange of medium several times per day. Explant location in the culture (submerged in medium or at the medium-gas interface) has a statistically marginal effect, with a slight preference for the interfacial location. Recent work suggests that survival is enhanced by a partial replacement circumfusion cycle, in which 30% to 50% of the culture medium volume is replaced with fresh medium at intervals of three hours.

Significance to Biomedical Research and the Program of the Institute: Cancer of the pancreas results in over 18,000 deaths per year in the United States, and the incidence is rising. Among the various types of cancer causing death in the country, it ranks sixth. In spite of this, there has been little

experimental work on cancer of the pancreas. There are no adequately developed animal models of this kind of cancer or adequate approaches to study in vitro transformation of pancreatic exocrine cells. This project is one of several initiated by the Biological Models Segment in order to develop animal models and experimental approaches to the study of the etiology and pathogenesis of this major form of human cancer.

Proposed Course: The best combinations of circumfusion culture conditions defined thus far maintain cultures for three to five days. To extend this viable period, analyses of the system-dependent factors noted above are continuing. Interactions among the conditions are probable, and experimental designs to detect such interactions are fundamental to the research plan. Major emphasis will be placed on determining the effects of culture medium supplements and osmolality adjustments and on long-term effects of variations of the partial replacement circumfusion sequence.

Date Contract Initiated: June 29, 1972

Current Annual Level: $109,285

WEST VIRGINIA UNIVERSITY (NO1-CP-43230)

Title: Relationships of Pituitary Hormones and Androgen on Prostate Metabolism

Contractor's Project Director: Dr. John A. Thomas

Project Officer (NCI): Dr. Douglas H. Janss

Objectives: The major objective of these investigations is to determine how pituitary hormones, such as prolactin, affect the growth and biochemical regulation of different lobes of the prostate glands. This study involves a comparison of several different species with regard to prostate physiology. Since the prostate gland is androgen dependent, these studies will be concerned with the interaction of these steroids and/or non-gonadotropic hormones. Particular emphasis will be placed upon the effect(s) that prolactin exerts upon testosterone metabolism. Further, the objectives will involve the role of 3'5'-adenosine monophosphate in the modulation of prostate gland growth. The results obtained from these studies may provide further insight into the biochemical processes involved in normal and abnormal growth of the prostate gland.

Major Findings: In vitro incubation of the guinea pig sex accessory glands (viz. prostate and seminal vesicle epithelium and muscle) with ovine prolactin caused significant reductions in the reductive metabolism of testosterone-H^3. This metabolic inhibition was most notable in homogenates rather than in tissue slices. The inhibition was also observed in the presence of TPNH or DPN. Bovine prolactin, under the same conditions, had no discernable effect on testosterone-H^3 metabolism. Testosterone caused an increase in the incorporation of ^3H-thymidine into various lobes of the rat prostate gland (ventral,

lateral, anterior, and dorsal). The specific activity of ^{3}H-DNA was increased by prolactin and/or testosterone treatment indicating an increase in the net synthesis of DNA. The pool size of radioactive thymidine nucleoside and nucleotide, however, remained unchanged. The in vitro incorporation of ^{3}H-thymidine into normal mouse sex accessory organ DNA was significantly increased by prolactin pre-treatment. In castrate mice, prolactin augmented the testosterone-induced increases in the weight of the prostate and seminal vesicles. This simultaneous treatment with prolactin and testosterone caused an augmentation in prostatic protein content, but not fructose levels (content or concentration). Normal mice pre-treated with prolactin failed to affect sex accessory organ weights or proteins. A ten-day period of castration caused a significant increase in the concentration of mouse prostate gland cAMP as well as cGMP.

Significance to Biomedical Research and the Program of the Insitute: - Incidence of prostate adenocarcinoma in the United Statws remains quite high, with approximately 30,000 to 40,000 cases diagnosed each year. The recent annual death rate due to this disease is about 15,000 to 20,000. Unfortunately, little is known about the etiology of this disease, and just what factors may be the most important insofar as the regulation of prostatic growth. Prostatic growth in mammals has not received very much attention, despite the incidence of human prostatic adenocarcinomas. There is a great need to discover a suitable animal model whereby cellular growth can be studied more thoroughly with the hope that such findings may be of value in gaining further insight into this disease in man. The sex accessory organs, such as the prostate, are not only androgen dependent, but their growth appears to be directly affected by non-gonadotropic pituitary hormones. This project will make an effort to elucidate some of the cellular events occurring in the prostate gland's cells following the administration of prolactin and other pituitary hormones. By examining the different lobes of the prostate gland from various laboratory animals, perhaps a most suitable animal model can be determined.

Proposed Course: Using different species of laboratory animals (e.g., mouse, rat, guinea pig, and dog), sex accessory organs (e.g., prostate and seminal vesicles) will be investigated with respect to their in vivo and in vitro biochemical responsiveness to prolactin. Efforts will continue to be devoted towards investigating prolactin's ability to increase steroidogenesis (e.g., dihydrotestosterone formation) in sex accessory organs. The role of cAMP (and cGMP) in prostatic steroidogenesis will also continue to be investigated. Finally, the studies will investigate the effects of prolactin on prostate gland nucleic acid metabolism.

Date Contract Initiated: June 28, 1972

Current Annual Level: $85,166

WRIGHT STATE UNIVERSITY (NO1-CP-43231)

Title: Cell Culture Development of Human and Guinea Pig Pancreas Cells

Contractor's Project Director: Dr. Robert Hay

Project Officer (NCI): Dr. Stuart Yuspa

Objectives: To establish reproducible techniques for cultivation of pancreatic exocrine cells. Such cultures can then be used for studies on chemical carcinogenesis.

Major Findings: Cell suspensions consisting of 60 to 90% pancreatic acinor cells can now be obtained on routine dissociation with trypsin-collagenase mixture followed by centrifugation through a bovine serum albumin solution. Three dimensional aggregates, formed during gyration of this cell suspension, can be seeded to culture plates giving rise to cultures of two dimensional colonial aggregates. Studies of colonies stained by the Mallory-Heidenhain method indicate that zymogen droplets lose characteristic appearance within 24 to 48 hours. By electron microscopy, zymogen-like droplets also are seen to change with time *in vitro* but can be detected even as late as 120 hours after aggregate plating. No other overt signs of fine structure impairment have been observed.

Attempts to stimulate protein synthesis by colonial aggregates at 72 hours after plating have not yet been successful. Screening experiments involving addition of insulin, secretin, gastrin, pancreozymin, glucagon, hydrocortisone, somatotropin, and various preparations of chicken embryo extract have been completed.

Response of three dimensional aggregates of pancreatic cells to pancreozymin has been demonstrated at 24 hours after dissociation. These cellular complexes release greater amounts of amylase upon stimulation. Furthermore, enhanced release of radioactivity from aggregates pulse labelled with tritiated leucine was also detected following addition of this hormone.

Significance to Biomedical Research and the Program of the Institute: The development of a model system for cultivation of exocrine pancreatic cells would provide a powerful tool for the study of carcinogenesis. Definition of the role played by essential metabolites and hormones in regulating cell proliferation and function in such a system would also contribute to basic understanding of acinar cell physiology.

Proposed Course: Studies on the effects of essential metabolites and hormones on DNA synthesis in colonial aggregates of pancreatic cells are planned or in progress. Efforts to define important factors regulating protein synthesis will now be confined to short-term study of 24 hour aggregates in suspension. Attempts to activate non-functional colonial aggregates of acinar cells will also be made by return to host animals. Such experiments will be performed using the diffusion chamber implantation technique.

Date Contract Initiated: June 28, 1972

Current Annual Level: $55,976

SUMMARY REPORT

BIOLOGY AND IMMUNOLOGY SEGMENT

July 1, 1973 through June 30, 1974

The program of the Biology and Immunology Segment includes the following areas of activity: *in vitro* carcinogenesis and immunology. The main goals of this Segment are (1) to develop fast and reliable assays to detect chemical carcinogens and (2) to learn how to use immunologic methods to prevent progression of the carcinogenic process.

In Vitro Carcinogenesis

At Biolabs, Inc., the primary objective is to study *in vitro* assay systems which would be used for identification of potential carcinogens and to use these systems to evaluate the role of chemical carcinogens in promoting (enhancing) viral carcinogenesis.

Diverse chemical carcinogens enhance the frequency of transformation by simian adenovirus, SA7. The effect of adding various chemical carcinogens at times after virus innoculation and cell transfer was determined. Cell survival was quantitated by cloning procedures and SA7 transformation by counting transformed foci growing under 0.5% agar overlay. The carcinogens methylmethanesulfonate (MMS), 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) and N-acetoxy-2-fluorenylacetamide (N-acetoxy AAF) caused maximal enhancement of SA7 transformation when added 2 hr. before, or 5 hr. after, virus addition. A slight increase was also observed when these chemicals were added 24 hr. after virus, but not at 48 hr. Alternatively, the polycyclic hydrocarbons benzo(a)pyrene (BP), 3-methylcholanthrene (3-MCA), and 7,12-dimethylbenz(a)-anthracene (DMBA) increased the viral transformation frequency only when added 4 to 24 hr. prior to virus, but inhibited transformation when added at 4, 8, 12, or 24 hr. after virus. In contrast to both of the above observations, enhancement by treatment of cells with caffeine or 6-acetoxy-benzo(a)-pyrene was maximal when chemical was added 5 hr. after virus, but was minimal or absent when added prior to virus. Thus, maximal chemical enhancement of adenovirus transformation depends upon the sequence of addition of virus and chemical.

Clones of transformed hamster embryo cells were characterized for (1) content of virus-specified antigens; (2) cloning capacity in soft agar; and (3) tumorigenicity in hamsters. All clones contained SA7 "T" antigen (detectable by complement fixation). There was no correlation between antigen levels and type of chemical pretreatment. Tumor transplant rejection data indicate that transformed clones derived from combined treatment contain transplantation antigens similar to those found in clones transformed by SA7 alone. All clones transformed by SA7, or by the interaction of chemical and virus, formed colonies in soft agar. The quantitative differences in cloning efficiency were not related to treatment. All clones were tumorigenic, but some selected from SA7 transformed cells that had been pre-treated with 3-methylcholanthrene, 7,12-dimethylbenz(a)anthracene, or benz(a)pyrene exhibited increased tumor incidence and shorter latent period compared to cells

transformed by SA7 alone. The degree of tumorigenicity could not be predicted by relative cloning efficiency in soft agar.

At Ohio State University the objective is to evaluate human cell culture systems as in vitro assays for chemical carcinogenesis. This includes identification of potential carcinogens, evaluation of the role of hormones as inhibitors or promoters of in vitro carcinogenesis and investigation of the role of nononcogenic and oncogenic viruses in promoting chemical carcinogenesis in human cells.

Alterations of growth characteristics including morphologic changes of human cells in culture have been obtained with several carcinogens. Such cultures possessed increased cell density, greater doubling potential, losses of contact inhibition, increased cloning efficiency and ability to grow under or in soft agar. These effects were enhanced by the addition of 17-β-estradiol or cortisone acetate.

Transformation of Detroit-550 cells by SV_{40} was enhanced by pretreatment with BP, MNNG, 4NQO and N-acetoxy-AAF but not by MCA or DMBA. In contrast to these results, promotion was not achieved with DMBA(0.5% μg/ml) or with MCA-(5.0 μg/ml). MCA significantly inhibited transformation when added 2, 6 or 24 hrs. after virus. DMBA also diminished transformation rates to a lesser degree when added after virus. The ability of these chemicals to induce damage to cellular DNA, alter its repair or influence scheduled DNA synthesis differs in Detroit-550 cells. These differences are reflected in their effects on transformation of human cells by SV_{40}.

Immunology

At the Trudeau Institute studies performed in collaboration with Dr. George Mackaness and Dr. Thomas Miller have indicated that method of preparation has a significant effect on the tumor suppressive properties of Mycobacterium bovis strain BCG. Preparations of bacteria prepared in the presence of detergent and then frozen are more effective in causing tumor regression than are preparations of bacteria grown in the absence of detergent and then frozen and dried. At Ivan Sorvall, Inc. (Rocky Mountain Laboratory) in collaboration with Dr. Edgar Ribi, Dr. Ischiro Azuma and Dr. Thomas Meyer, it was found that a trehalose-mycolic ester was essential for BCG mediated regression of established intradermal tumors in guinea pigs. At the Oak Ridge National Laboratory in collaboration with Dr. Michael Hanna, it was found that during BCG mediated eradication of microscopic lymph node metastases, histiocytes are found in close contact with dying tumor cells.

At the University of Illinois in collaboration with Drs. Dray and Paque it was found that the ability of immune lymphocytes to recognize tumor antigens can be transferred to normal lymphocytes in vitro with extracts of lymphoid tissues from immunized guinea pigs.

Three contracts are devoted to study the detection, significance and usefulness as tumor markers of two tumor associated molecules: CEA of human and alpha-fetoglobulins of rats and guinea pigs. CEA arises in patients suffering from a wide variety of cancers of epithelial origin, especially cancer

of the colon; alpha-fetoglobulin increases in the circulation of patients and animals suffering from primary hepatomas. The contract exploring rapid screening techniques for the presence of CEA is being phased out because it is now clear that rapid screening of large numbers of people is not feasible in light of present day knowledge (Mallory Institute). This contract has been instrumental in defining the limits of usefulness of CEA assay in diagnosis of cancer and has received international recognition for its pioneering work. Because everyone seems to have low levels of CEA normally present in serum, we have initiated an immunochemical study of the "normal" CEA to permit comparison with the "abnormal" CEA in cancer patients. Preliminary results indicated that the two forms of CEA may differ significantly from each other (Scripps Clinic and Research Foundation). Studies on rat alpha-fetoglobulins showed that increased production of alpha-fetoglobulin is not a necessary consequency of malignant transformation of liver cells; on the other hand, alpha-fetoglobulin appears in the serum of rats within one week after feeding an oral hepatocarcinogen. The increase is produced by a dose of carcinogen that produced no morphological changes in the liver and that is insufficient to induce hyperplastic or neoplastic changes (University of California).

The function of macrophages was studied in two contracts. In one the in vivo and in vitro effects of chemical carcinogens on the synthesis of complement components were studied. Nitrosamines that inhibit in vitro C4 and C2 synthesis within 24 hours after in vivo administration do not inhibit the in vitro production of these components if the carcinogen is administered in vitro. Chemotaxis of macrophages was also inhibited by in vivo injection of nitrosamines (Childrens Hospital Medical Center). In another contract, it was found that chemotaxis of human macrophages is greatly reduced in about one-half of patients suffering from cancer (Duke University Medical Center).

Studies based on the fact that some carcinogens are allergens and haptens are being done at four contract sites. A contract with Becton, Dickinson Research Center had the goal of determining which routes of exposure would cause an immune response to chemical carcinogens. It was found in confirmation of previous studies that intradermal injection or topical application of carcinogen in guinea pigs produced immunological hypersensitivity. Skin hypersensitivity was never obtained by the aerosol route not only with carcinogens but with the potent sensitizer 1-fluoro-2,4-dinitrobenzene given as a hapten or protein conjugate. This contract was terminated on December 21, 1973. Progress has been made in finding suitable coupling agents for carcinogens so that they become immunologically active in vitro (University of Texas at San Antonio) and in vivo (Brandeis University). In vitro tests are now being developed that may permit the detection of cellular immune reactivity to carcinogens as a measure of previous exposure to these agents. Antibodies have been produced to several carcinogens that may make it possible to develop tests of humoral immunity to detect prior exposure to the carcinogen. The principle of this kind of detection system has been established with non-carcinogens at Brandeis University by showing that nicotine and cotinine can be detected in the serum of smokers by use of specific antibodies to these chemicals. In addition, antibodies have been made to adriamycin a promising chemotherapeutic agent which is suspected of also being a carcinogen. At Case Western Reserve University animals are rendered immunologically tolerant to a chemical carcinogen and then tested for susceptibility to

tumorigenesis by that carcinogen. Tolerance is induced by oral feeding of carcinogen. Oral feeding of carcinogens so far has produced no tumors in guinea pigs. Intravenous infusions of DMBA in fat emulsion has produced mammary tumors in guinea pigs. Procedures have been devised that regularly produce tumors in guinea pigs so that the final phase of this project can now be initiated.

At Temple University guinea pigs whose skin has been painted with DMBA for more than one year have developed squamous cell carcinoma resembling the human disease. They have also developed melanotic lesions. These carcinomas are focal lesions that do not obliterate the melanotic lesions. The melanotic lesions now have two components: melanocytes and macrophages which have ingested melanocytes. In the painted area, the animals are developing small brown macules that may represent the first proliferation of melanocytes. Histology has not yet been done to determine whether these animals have malignant melanoma. Development of this disease in guinea pigs resembles that seen in humans. When all stages of development of the disease in guinea pigs have been observed and studied the next phase of this project will be initiated; namely, to determine whether there are stages in the carcinogenic process where immunological maneuvers can prevent progression of the disease.

Studies are underway at the University of Utah to determine the effects of chronic treatment with cyclophosphamide, methotrexate, azathioprine, prednisolone, azathioprinze plus prednisolone, and antilymphocytic serum on benzopyrene and ultraviolet (UV) skin carcinogenesis in mice and to investigate the immunological aspects of UV carcinogenesis in mice. Appropriate doses of benzopyrene and UV light to use in studies combining treatment with these carcinogens and immunosuppressive drugs have been defined. Dose and regimen for each of the immunosuppressive agents which permit chronic drug administration without excessive toxicity have been determined. The effects of the immunosuppressive drugs on skin allograft rejection have been investigated. It has been found that tumors induced by UV light exhibit an unusually high degree of antigenicity, and that they are not detectably cross reactive when tested by transplantation techniques. The findings on the antigenicity of UV-induced tumors provide a unique opportunity to investigate the mechanism which enables highly antigenic tumors to escape immunologic destruction in the primary host.

CONTRACT NARRATIVES

BIOLOGY AND IMMUNOLOGY SEGMENT

July 1, 1973 through June 30, 1974

AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory) (NO1-CP-40013)

Title: NCI-AEC Carcinogenesis Program

Contractor's Project Director: Dr. Francis T. Kenney

Project Officer (NCI): Dr. Allen Heim

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis.

BECTON, DICKINSON AND COMPANY RESEARCH CENTER (NO1-CP-12168)

Title: Carcinogens as Allergens: Detection of Exposure to Carcinogens by Cell-Mediated Immunologic Reactions to the Carcinogens

Contractor's Project Director: Dr. Jerry J. Tulis

Project Officer (NCI): Dr. Herbert J. Rapp

Objectives: To determine whether exposure of experimental animals to known carcinogens via various routes will result in the elaboration of in vivo (delayed hypersensitivity) and in vitro (lymphocyte transformation or macrophage inhibition) immunologic reactions. The ultimate goal of the program is the development of in vitro screening procedures to determine the potential hazards to man from exposure to environmental carcinogens. Ancillary objectives include determinations of the retention, distribution, and clearance of carcinogens in aerogenically exposed animals.

Major Findings: Aerosol exposure of guinea pigs to varied doses of the carcinogens benzo(a)pyrene, 9,10-dimethyl-1,2-benzanthracene, 20-methylcholanthracene, benz(a)anthracene, and 4-nitropyridine-N-oxide and to the potent sensitizer 1-fluoro-2,4-dinitrobenzene, both as a hapten and protein conjugate, failed to elicit dermal delayed sensitivity responses. Findings indicate that lack of response was not due to tolerance, chemical alteration of the carcinogen by the solvent carrier, or absence of in vivo conjugation by the haptenic carcinogen to form an allergen. Aerosol retention and distribution studies revealed that about 15% of tritiated benzo(a)pyrene is retained in guinea pig lungs immediately after aerogenic exposure. Clearance rates appear to be 75% after 12 hours and 86% after 7 days. Preliminary evidence shows initial concentrations of radioactivity to occur in the inferior lobes, trachea, and carina of the trachea as compared to the superior lobes. Rapid clearance appears to take place from the trachea as compared to the lobes of the lung.

The in vitro preparation by UV or isocyanate coupling of benzo(a)pyrene-skin conjugate yielded dermal sensitivity, macrophage migration, and lymphocyte blastogenesis responses.

Significance to Biomedical Research and the Program of the Institute: Clear evidence for the development of delayed sensitivity to certain carcinogens acting as haptens when administered by dermal routes and absolute lack of contact sensitivity when administered by the respiratory route, provide meaningful information related to environmental exposure of man to carcinogens. Preliminary data on retention, distribution, and clearance of aerosolized carcinogen provides heretofore unavailable quantitative information directly related to future aerobiologic studies.

Proposed Course: This contract was terminated on December 21, 1973.

Date Contract Initiated: June 22, 1971

Current Annual Level: $0

BIOLABS, INC. (NOI-CP-33282

Title: In Vitro Study of the Nature of the Interaction Between Chemical and Viral Carcinogens

Contractor's Project Director: Dr. Bruce C. Casto

Project Officer (NCI): Dr. Joseph A. DiPaolo

Objectives: The specific objective of this contract is to study the process whereby chemical carcinogens promote viral oncogenesis and to determine if oncogenic and non-oncogenic viruses may similarly promote oncogenesis by known chemical carcinogens. This contract is part of a program to develop in vitro screening methods for detection of potential carcinogenic hazards, either viral or chemical.

Major Findings: This study has established that treatment of cells with chemical carcinogens will increase the frequency of viral transformation in vitro. Over 40 chemicals, including polycyclic hydrocarbons and alkylating agents, have been tested for their capacity to enhance viral (Simian adenovirus, SA7) transformation of hamster cells, induced single-strand breaks in cell DNA, and stimulate unscheduled DNA (repair) synthesis.

Carcinogenic polycyclic hydrocarbons enhanced adenoviral transformation, but the non-carcinogens did not. Enhancement was demonstrated by MNNG, Ac-AAF, MMS and MAM-Ac, but not by DEN or DMN. Enhancement of SA7 and SV40 transformation was also demonstrated in rat embryo and human lung cell cultures, respectively, by certain chemical carcinogens.

The enhancement by BP, 3-MC and 7,12-DMBA was demonstrable if hamster cells were pretreated for 4, 8, 12 or 24 hours prior to virus, with an 8 to 24 hour incubation optional for BP and 3-MC, whereas a 4 to 24 hour pretreatment was equally effective for 7,12-DMBA. Addition of any of these three agents up to 48 hours after virus inhibited transformation. MNNG, Ac-AAF, and MMS caused

enhancement when added either 2 hours before, or 5 to 24 hours after, virus addition. In contrast to both of the above, maximal enhancement was observed when caffeine and 6-acetoxy-BP were added after virus, but little or no enhancement was shown when added prior to virus.

Maximal enhancement by MMS was observed when virus was added immediately after treatment; addition of virus beyond 12 hours after MMS treatment caused no enhancement. By comparison, enhancement by 7,12-DMBA persisted for at least 72 hours, but maximal enhancement was still observed when virus was added immediately following treatment.

Treatment of cells with benz(a)anthracene (BA) or 7,8-benzoflavone (ANF) inhibited cell death and enhancement by BP or 7,12-DMBA when added simultaneously to hamster embryo cells. Both ANF and BA reduced the toxicity of 3-MC, but had less effect on the enhancement of viral transformation by 3-MC. The protective effect of BA and ANF against BP and 7,12-DMBA was essentially gone by 24 hours after treatment, as well as the inhibition of enhancement. At several concentrations of BP and 7,12-DMBA where the toxicity of the chemical was decreased by BA or ANF, the actual number of SA7 foci increased several fold.

All compounds which were shown to induce single-strand DNA breaks, and/or stimulate cell DNA repair synthesis, enhanced transformation by SA7. However, enhancement was not restricted to chemicals which cause either of the above, since both DB(a,h)A and DB(a,c)A stimulate SA7 transformation, but do not produce demonstrable DNA breaks or repair synthesis.

Approximately 40 transformed clones, arising from the interaction of chemical and virus, and 17 clones transformed by virus alone have been examined for: (1) cloning capacity in soft agar; (2) content of viral-specified antigens; and (3) tumorigenicity in hamsters. These parameters were measured to determine if the interaction with chemical had introduced new properties that were not produced by virus acting alone. Although quantitative differences in the cloning capacity were found, these could not be related to the type of chemical pretreatment. All clones were tumorigenic, but the degree of tumorigenicity could not be predicted by the relative cloning efficiency in soft agar. All of the clones were positive for SA7 "T" antigen. However, there was no correlation between antigen levels and chemical pretreatment. Some SA7 clones, selected from cells treated with 3-MC, BP, or 7,12-DMBA exhibited an increased tumor incidence and shorter latent period when compared to cells transformed by SA7 alone.

Significance to Biomedical Research and the Program of the Institute: In view of the various hypotheses concerning chemicals, viruses, and cancer, and due to the inherent difficulties in studies in the intact animal, it is advantageous to study the various interactions in a controlled *in vitro* system. This would establish not only the influence of oncogenic and non-oncogenic viruses in promoting chemical carcinogenesis, but also the role of chemicals in promoting viral carcinogenesis. Sensitive quantitative assays, such as those developed under this contract, may be used to detect potential carcinogenic or mutagenic chemicals in the environment.

Proposed Course: Diverse chemicals that are important to the human environment, including pesticides, herbicides, and industrial chemicals, will be considered for their ability to influence viral transformation. The nature of the persistence and inhibition of enhancement by chemical carcinogens will be the subject of further studies. An attempt will be made to correlate the persistence of enhancement with DNA breaks, and where such breaks are not found an attempt will be made to determine if the chemical carcinogen remains bound. The factors influencing the quantitative transformation by polycyclic hydrocarbons will be determined by using compounds known to influence the toxicity of these carcinogens as well as by altering the type of cells at risk, the concentration of the chemical compound, and also the length of time the cells are exposed. An evaluation will also be made to determine if the phenomenon of enhancement observed will occur with other types of host cells and viruses. The influence of different types of viruses on the frequency of transformation by chemicals will be explored using a quantitative focus assay that does not require the use of feeder cells. Cells transformed by the combined action of chemical and virus will be characterized and compared to cells transformed by virus alone. Preliminary evidence, from soft agar cloning experiments and tumorigenicity in animals, indicate that the chemical may contribute additional properties to the cells as well as stimulate viral transformation. Thus, the clones that have been transformed will be studied for antigenic changes (especially viral-specified antigens) and chromosome alteration with the hope that these parameters may be correlated with an altered tumorigenicity.

Date Contract Initiated: June 9, 1971

Current Annual Level: $232,804

BRANDEIS UNIVERSITY (NO1-CP-23243)

Title: Production and Detection of Antibodies to Chemical Carcinogens and Other Small Molecules

Contractor's Project Director: Dr. Helen Van Vunakis

Project Officer (NCI): Dr. Herbert Rapp

Objectives: To obtain antibodies that react specifically with chemical carcinogens and other small molecules of interest in cancer research. The antibodies will be used as reagents to develop immunoassays capable of detecting the corresponding haptens in body fluids and tissues. Haptens attached to appropriate carriers will be used as antigens to detect presence of antibodies in the serum of animals and humans. These reagents will be tested for their suitability for the detection of exposure to environmental agents that may be related to cancer causation.

Major Findings: Sensitive and specific radioimmunoassays for nicotine and one of its major metabolites, cotinine, have been developed. Analyses of physiological fluids from smokers indicate that there is little or no correlation between smoking histories and the cotinine and nicotine levels in their

plasma and amniotic fluid. In contrast to nicotine which is rapidly metabolized, cotinine has a plasma half-life of approximately 20 hours. Since cotinine persists in the plasma for a longer period than the parent compound, the determination of cotinine levels should prove more informative in epidemiological studies of smoking-related illnesses. The radioimmunoassays have been used to monitor the oxidation of nicotine to cotinine by NADPH and oxygen-dependent oxidases present in rabbit liver extracts. The results obtained in the radioimmunoassays were confirmed by isolation and quantitation of cotinine and nicotine from the enzymatic reaction mixtures by high-pressure liquid chromatography. A radioimmunoassay for another metabolite, γ-(3-pyridyl)-γ-oxo-N-methylbutyramide, has been developed and is being applied to the analysis of physiological fluids.

Antibodies to benz(a)pyrene (BP) and 7,12-dimethylbenzanthracene (DMBA), produced in rabbits, have been characterized and radioimmunoassays for these polycyclic hydrocarbons (PCH) developed. With BP, the link to the carrier used for immunization was through the 1- or 6-positions, while for DMBA a derivative substituted in the 7-position was used. Structurally similar PCHs cross react to different extents with each antibody. It is possible, however, to determine levels of one compound (in a mixture of related compounds) by analyzing the mixture with two antisera of different specificities.

A radioimmunoassay for adriamycin, a drug which shows considerable promise in cancer chemotherapy, has been developed. Pharmacokinetic studies on rabbits, monkeys, and 20 patients with different types of cancer have been carried out. The assay is sensitive and permits levels of the drug to be determined in plasma and urine samples taken one week after administration of the drug to patients. This assay is also applicable in estimation of levels of daunomycin, a drug closely related to adriamycin but used in patients with different types of malignancies.

Significance to Biomedical Research and the Program of the Institute: This work may provide a way to determine the degree to which individuals in different environments have been exposed to known chemical carcinogens. The immunologic approach does not require that the carcinogen be present in the individual at the time of testing. A metabolite, which is longer lived in physiological fluids than the parent compound and/or which may be responsible for the biological effect, may provide a more reliable base for epidemiological studies. Demonstration that different groups are at risk may provide the impetus to remove certain agents from the environment. In the case of adriamycin, it may be possible to relate its toxicity to plasma levels and design regimens for individual patients which would permit the drug to be used for chemotherapeutic purposes under conditions which minimize its toxic side effects. An assay which facilitates studies on its mechanism of action will help to define the circumstances under which it might also be carcinogenic.

Proposed Course: To proceed with the objectives as stated. Rabbits or other suitable animals will be immunized with the chemical substance of interest attached to a carrier that promotes the antigenic activity of the agent. Among the agents to be used are polycyclic hydrocarbons, aflatoxins, other molecules which may be carcinogens or procarcinogens.

Date Contract Initiated: April 20, 1972

Current Annual Level: $97,507

CALIFORNIA, UNIVERSITY OF (at La Jolla) (NO1-CP-33403)

Title: Significance and Relationship of Fetoglobulins to the Induction of Hepatomas by Chemical Carcinogens

Contractor's Project Director: Dr. Steward Sell

Project Officer (NCI): Dr. Tibor Borsos

Objectives: The objective of this contract is to develop a sensitive new method to study the relationship between the induction of primary hepatomas and the appearance and significance of fetoglobulins.

Major Findings: Rat alpha$_1$-fetoprotein (α_1F) has been isolated and a competitive binding radioimmunoassay sensitive to 1 ng developed. Normal adult rats have a serum α_1F concentration of 0.03 µg/ml. Secretion of α_1F occurs following mitosis of neonatal liver cells, of adult liver cells following partial hepatectomy or chemically induced hepatitis, and of fetal liver cells stimulated to divide *in vitro*. During gestation, α_1F is produced by dividing fetal liver cells. There is rapid equilibration from fetus to mother with a fetal serum concentration of 5,000 µg/ml and maternal concentrations of 200 µg/ml at term.

Transplantable hepatoma (liver cancer) cells also produce α_1F. The serum α_1F concentration may be as high as 15,000 µg/ml. However, the sera of some animals bearing transplantable hepatomas do not have elevated serum α_1F concentrations. The capacity of a given hepatoma to produce α_1F is related to growth rate, degree of differentiation and karyotype.

As an α_1F producing hepatoma grows, the serum α_1F concentration increases. Removal of the tumor by surgery results in a rapid fall in serum α_1F concentration with a half-life of 24 hours. The presence of occult metastases may be determined by rising α_1F concentrations in rats whose primary implanted tumor has been amputated. This provides a model for therapy of minimal residual metastatic tumor. Hepatomas growing in immunosuppressed hosts enlarge more rapidly, contain more cells in mitosis and produce more α_1F. Not only does the serum α_1F concentration rise at a more rapid rate, but also there is more α_1F per gram of tumor. This indicates that α_1F is synthesized by dividing tumor cells, and not presumably by quiescent tumor cells.

Elevations of serum α_1F concentrations occur within one week of feeding oral hepatocarcinogens. These elevations are produced by doses of carcinogen that produce no detectable morphologic changes in the liver and are insufficient to induce hyperplastic or neoplastic changes. The serum concentrations of α_1F in rats fed carcinogens remain elevated for up to 20 weeks after the carcinogen is removed from the diet.

Radioimmunoassays have also been developed for guinea pig and human α_1F.

Significance to Biomedical Research and the Program of the Institute: The primary goal of the Carcinogenesis Program is to prevent cancer in man by identifying carcinogenic compounds and formulations that may affect humans, as well as by identifying the mechanism of action of known carcinogens. Both these approaches require extensive bioassay and other analytical technologies, as well as a continuing vigorous program for refinement and innovation of the same technologies.

This contract is suited to the goal-oriented program of the Biology and Immunology Segment of the Carcinogenesis Program. It would apply immunochemical techniques to the detection and to the study of the significance of alpha-fetoglobulins as a consequence of chemical carcinogenesis. Furthermore, it has direct applicability to prospective and follow-up studies of cancer patients. The test may ultimately prove useful in monitoring workers who are exposed to carcinogens.

Proposed Course: In developing a method to study the relationship between the induction of primary hepatomas and the appearance and significance of fetoglobulins, the contractor will induce primary hepatomas with various chemical carcinogens in selected animals (e.g., guinea pigs), characterize by histologic, biochemical and other means the changes occurring in the liver and study the relationship of fetoglobulins to the development of the liver lesions.

Date Contract Initiated: June 28, 1972

Current Annual Level: $148,619

CASE WESTERN RESERVE UNIVERSITY (NO1-CP-23220)

Title: Specific Immunological Unresponsiveness to Chemical Carcinogens and Its Influence on Tumorigenesis

Contractor's Project Director: Dr. Jerome Pomeranz

Project Officer (NCI): Dr. Herbert Rapp

Objectives: To determine whether immunological responses to chemical carcinogens qualitatively or quantitatively influence tumorigenesis.

Major Findings: Delayed contact hypersensitivity to dimethylbenz(a)anthracene (DMBA) was induced in guinea pigs by foot-pad injection of the chemical in adjuvant and immune tolerance to contact sensitization was produced by feeding or infusion of the chemical. Since adjuvant is known to modify immune responses to tumors, topical methods of contact sensitization have also been devised. Feeding large amounts of DMBA to guinea pigs did not result in tumorigenesis, but mammary tumors have appeared in recipients receiving large doses of DMBA in fat emulsion by intravenous infusion. Contact sensitization

to a second antigen, picryl chloride was not prevented by feeding, infusion, or injection of DMBA, but the intensity of the response was significantly diminished. Methods for inducing contact sensitization to DMBA have been successfully applied to mice. Finally, procedures for regularly inducing tumors in guinea pigs by subcutaneous injection of the carcinogen have been devised.

Significance to Biomedical Research and the Program of the Institute: The demonstration that guinea pigs and mice may be contact sensitized to carcinogens provides a means of measuring a parameter of cell mediated immunity to these compounds. The induction of contact tolerance to these chemicals permits experiments in which the effect of absent cell mediated immunity to carcinogens may be studied. This project will attempt to correlate the presence or absence of specific immunity to a carcinogen with the compound's capacity to induce a malignant tumor.

Proposed Course: Guinea pigs that are immunologically unresponsive or contact sensitized to DMBA are available. Methods for uniformly inducing tumors with this compound, which do not regularly sensitize or tolerize the recipient, have also been devised. Responsive or unresponsive animals are being evaluated for the effect of immunity to the carcinogen on their susceptibility to oncogenesis with the same compound.

Date Contact Initiated: February 15, 1972

Current Annual Level: $109,171

CHILDREN'S HOSPITAL MEDICAL CENTER (NO1-CP-33270)

Title: Effects of Carcinogens on the *In Vitro* Synthesis of Complement Components

Contractor's Project Director: Dr. Harvey R. Colten

Project Officer (NCI): Dr. Tibor Borsos

Objectives: The major goals of this contract consist of studies of the effects of selected chemical carcinogens (primarily, nitrosamines) on normal macrophage function. These macrophage functions include biosynthesis of the second (C2) and fourth (C4) components of complement, total protein synthesis, cell surface receptors and response to soluble mediators of macrophage mobility (migration inhibition factor and chemotactic factor).

Major Findings: Two carcinogenic nitroso-compounds, diethylnitrosamine (DEN) and dimethylnitrosamine (DMN) were highly effective inhibitors of complement (C2 and C4) biosynthesis *in vivo* but were ineffective *in vitro*. For example, *in vivo* administration of DMN to guinea pigs at a dose of 30 mg/kg results in 85-95% inhibition of C2 and C4 synthesis, whereas *in vitro*, even at a concentration of 10^{-3}M, this compound had no effect. Likewise DEN significantly inhibited (>80%) C2 and C4 biosynthesis when administered to guinea pigs but had no effect *in vitro* on complement production by normal guinea pig cells or

tissues. In addition, chemotaxis of macrophages obtained from guinea pigs fed DEN was inhibited more than 50% as compared to control macrophages. Moreover, generation of chemotactic factor was significantly less in serum harvested from animals fed DEN; suggesting an effect of DEN on serum factors as well as on the macrophages.

Diphenylnitrosamine, a non-carcinogenic nitroso-compound inhibited complement synthesis in vitro but not in vivo. In fact, in vivo administration of diphenylnitrosamine resulted in enhanced biosynthesis of C2 and C4. At the present time it is not clear whether this compound is a specific or non-specific stimulator of complement synthesis.

Significance to Biomedical Research and the Program of the Institute: This work provides insight into a number of basic alterations in effector cells of the immune system resulting from exposure to chemical carcinogens. In particular, preliminary findings suggest that several chemical carcinogens inhibit macrophage functions including complement biosynthesis, total protein synthesis, and possibly membrane dependent macrophage activity. Some of the compounds are effective in vitro whereas others, such as DEN and DMN are only active in vivo. The project has significance for the control of human cancer inasmuch as the findings suggest a broader role of carcinogens in the etiology and pathogenesis of tumors. Hence, the major benefit of this program is in the area of defining the causes of cancer which, in turn, may lead directly to practical changes in therapy. Moreover, the alterations in the immune capacity of experimental animals during administration of chemical carcinogens preceded the appearance of frank tumor. Such changes, therefore, may be useful in monitoring the potential risk of tumor development and/or the efficiency of a particular mode of therapy. Extension of these studies to an analysis of basic aspects of cellular immune function in humans with cancer can be accomplished with well-defined techniques currently employed in a number of laboratories. The animal experimentation in this contract work permits a more rational choice of areas in immunology to study in a similar analysis of human tumors. Such a choice will maximize the probability of findings that will achieve the overall goals of cancer prevention and treatment.

In addition, the contract provides a relatively inexpensive, rapid way to examine the early events in carcinogenesis with particular emphasis on the effect of carcinogens on protein synthesis of immunologically active proteins.

Proposed Course: The findings of several effects of DEN on macrophage function in addition to its effect on complement synthesis require expansion of future work to include the following: (a) To identify additional quantitative and qualitative changes in macrophage function associated with in vivo administration of chemical carcinogens; (b) To determine whether these alterations in macrophage function affect immunologic competence during induction of chemical carcinogenesis; (c) To determine, using non-carcinogenic hepatotoxins in vivo, whether changes in macrophage function and immune competence are specific for carcinogenic agents.

Date Contract Initiated: June 1, 1971

Current Annual Level: $93,145

DUKE UNIVERSITY MEDICAL CENTER (NO1-CP-33313)

Title: Response of Peripheral Blood Monocytes from Patients with Neoplastic Disease to Chemotactic Factors

Contractor's Project Director: Dr. Ralph Snyderman

Project Officer (NCI): Dr. Edward Leonard

Objectives: (1) To determine whether impaired delayed cutaneous hypersensitivity responses in cancer patients are related to deficient blood monocyte chemotactic responsiveness to chemotactic factors; (2) To quantitate chemotactic lymphokine production by peripheral blood lymphocytes in cancer patients; and (3) To determine the effect of BCG and/or tumor removal on monocyte chemotactic responsiveness and lymphokine production in patients with cancer.

Major Findings: (1) Responsiveness of peripheral blood monocytes to a chemotactic lymphokine is depressed (mean responses more than 2 S.D. below normal) in approximately 65% of patients with cancer. All patients with depressed chemotactic responsiveness also had depressed delayed cutaneous hypersensitivity to ubiquitous antigens. The data suggests that depressed delayed hypersensitivity in patients with cancer can result, at least in part, from defective monocyte chemotaxis. (2) Patients with recurrent malignant melanoma were immunized with BCG then rechallenged with intralesion injections of BCG. Prior to immunotherapy, 60% of patients had depressed monocyte chemotaxis. Following BCG, deficient chemotaxis was found in only 20% of patients while 40% of patients had developed enhanced chemotactic responses (mean chemotactic responses >2 S. D. above normal).

Significance to Biomedical Research and the Program of the Institute: Evidence suggests that a function of the immune system is to protect the host against the development of neoplasms. By evaluating cellular immune effector function in patients with cancer it should be possible to determine if the inability of a host to destroy a neoplasm is due to a defect in the effector limb of the immune response. This information is relevant to an understanding of the mechanisms of tumor formation and subsequent development of methods for prevention, treatment, and possibly early diagnosis of cancer. The finding of depressed monocyte chemotactic responsiveness in patients with cancer and the ability of BCG immunotherapy to enhance chemotaxis in many patients may explain one mechanism by which BCG affords protection to cancer patients. Moreover, it will be determined if quantitation of monocyte chemotaxis provides a prognostic index for cancer patients and perhaps a guide for the effectiveness of various immunotherapeutic measures.

Proposed Course: Quantitative measurements will be made on the response of peripheral blood monocytes to complement derived factors as well as to those produced by lymphocytes in response to specific antigens or mitogens. Chemotactic responsiveness will be correlated with delayed cutaneous hypersensitivity, tumor regression and patient survival. The effect of BCG immunotherapy on monocyte chemotaxis will be studied further. The prognostic value of monocyte chemotactic responsiveness will be determined. Quantitation of

lymphokine production will be determined for normals and for patients with cancer.

Date Contract Initiated: June 25, 1973

Current Annual Level: $75,838

ILLINOIS, UNIVERSITY OF (NO1-CP-23205)

Title: Transfer of Tumor Immunity by Cell-Free Extracts of Immune Lymphoid Cells

Contractor's Project Directors: Dr. Sheldon Dray
Dr. Ronald E. Paque

Project Officer (NCI): Dr. Berton Zbar

Objectives: To develop an immunotherapy model for cancer using sub-cellular components of immune lymphoid cells to transfer cellular immunity to non-immune lymphoid cells.

Major Findings: RNA extracts prepared from lymphoid tissues of strain-2 guinea pigs immunized against two antigenically distinct hepatomas, Line-1 and Line-10, can transfer specific sensitivity for each tumor to nonsensitive guinea pig peritoneal exudate cells (GP-PEC) *in vitro* as assessed by the cell migration inhibition assay. This work has been published recently. Nineteen local passive transfer experiments in which RNA-treated lymphoid cells (10^7) were injected locally and tested with the antigen (PPD, Line-1 antigen, or Line-10 antigen). The experimental protocols were varied to include lymph node, spleen, and peritoneal exudate cells and in some experiments epinephrine was included in the incubation mixture. No positive skin tests were observed; no inhibition of tumor growth was observed.

In the past seven months work was begun on the isolation and identification of a component of RNA extracts from lymphoid tissues of guinea pigs capable of transferring tumor specific sensitivity to peritoneal exudate cells (PEC). Data indicate that the active moiety capable of transferring Line-1 or Line-10 sensitivity to PEC resides between the 4S and 18S peaks. While resolution, isolation, and definition of the active moiety remains a crucial problem, experimental thrust is being shifted directly to *in vivo* experiments.

The results of experiments on the treatment of Strain-2 guinea pigs with lysates from rhesus monkeys xenografted with Line-10 hepatoma cells can be summarized as follows:

TREATMENT[1]	ANIMALS	SURVIVAL TIME (DAYS)
5 x 10^6 Line-10 I.D. + 0	3	31, 35, 37
5 x 10^6 Line-10 I.D. + Leucocyte lysates	6	85, 95, 104 >252, >210, >222

[1]Injected I.V., I.P., and subcutaneously with lysates on days 1, 5, 8, 17, 19 and 24.

Significance to Biomedical Research and the Program of the Institute: Intra- and interspecies conversions of immunocompetent cells with sub-cellular components; namely, RNA or fractions thereof, have basic significance for immunology and biology in general and several eventual clinical applications. For example, manipulation of the immune response with RNA could present a model for a clearer understanding of the relationships between humoral antibody synthesis and cellular immunity.

Proposed Course: It is planned to investigate the feasibility of various experimental procedures for the transfer of tumor specific immunity in inbred guinea pigs. Thus far the conditions used for local passive transfer have not been successful. Major efforts will be directed toward systemic transfer which can be approached in two ways: (1) injection of RNA treated lymphoid cells into guinea pigs; (2) injection of RNA extracts alone into guinea pigs.

It is planned to further document the activity of the RNA extract to a fraction of about 8-12S and also to prepare RNA on cesium chloride gradients by a new method which is purported to yield a better preparation of RNA which is free of DNA and protein.

The monkey to guinea pig transfer experiments will be continued, using both the leucocyte cell lysates and RNA. Since the leucocyte cell lysates have given very promising results, major efforts will be directed toward the use of these lysates. In these experiments the guinea pigs are challenged with a lethal dose of tumor cells and then given injections of leucocyte lysates. If the next set of experiments are successful, additional experiments will be planned immediately to assess the specificity of the lysates with respect to the two tumors and possible nonspecific effects will be assessed by using lysates from unimmunized monkeys.

Experiments directed toward assessing the mechanism whereby subcellular fractions of leucocytes have their effect on conferring immunity on non-sensitive cells are planned. Wherever possible the tumor system will be used in these studies; however, in some situations it may be necessary to use non-tumor antigens as models, since results with both tumor and non-tumor antigens appear to be parallel. The assessment of the mode of action will

require the use of metabolic inhibitors to assess the requirements for protein synthesis, DNA synthesis or RNA synthesis in the cells which are converted with sub-cellular extracts. Finally, the possibility that lymphoid cell populations may secrete molecules which have anti-tumor activity will be explored in a pilot study.

Date Contract Initiated: February 1, 1972

Current Annual Level: $115,000

MALLORY INSTITUTE (NO1-CP-33264)

Title: Detection of Carcinoembryonic Antigens in Humans

Contractor's Project Director: Dr. Norman Zamcheck

Project Officer (NCI): Dr. Tibor Borsos

Objectives: To develop techniques that will permit the identification of populations at risk to cancer and the monitoring of patients undergoing therapy. Specifically, the techniques should permit the detection in serum or plasma of carcinoembryonic antigen (CEA) and related antigenic markers of primary and/or metastatic cancer.

Major Findings: Combining solid phase adsorbents, electrophoresis and radioisotopes, a method has been developed which may be applied to measurement of tumor markers in large numbers of specimens of serum or plasma. The use of affinity chromatography media coupled with antibodies fixed in electrophoretic media to which macromolecules are electrically transported, combined with the sensitivity and reproducibility of isotopic measurement, provides a potentially useful method for the rapid detection of tumor markers in large numbers of serum samples. This solid phase radioimmunoelectrophoretic method presently permits measurement of a range of 10 to 160 nanograms of CEA per ml of serum.

Significance to Biomedical Research and the Program of the Institute: The development of the solid phase radioimmunoelectrophoretic method for detection and monitoring of cancer patients falls under objectives to improve methods and programs for cancer detection, diagnosis and screening.

Proposed Course: The improvement and standardization of the solid phase radioimmunoelectrophoretic method and its application to the rapid screening of large numbers of patient samples for detection of early and metastatic cancer, and the monitoring of patients undergoing therapy.

Date Contract Initiated: June 1, 1971

Current Annual Level: $113,941

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NO1-CP-43276)

Title: In Vitro Study of the Nature of Interaction between Chemical and Viral Carcinogens

Contractor's Project Director: Dr. David Yohn

Project Officer (NCI): Dr. Joseph DiPaolo

Objectives: To determine whether known or potential chemical carcinogens as specified will cause morphologic and neoplastic transformation of selected human cells in vitro. To determine if these processes require potentiators such as hormones, DNA repair inhibitors or enzymatic activators of polycyclic carcinogens. To determine whether the selected known or potential carcinogens enhance transformation of human cells by known tumor viruses SV_{40} and Feline sarcoma virus (FSV).

Major Findings: Alterations of growth characteristics including morphologic changes of WI-26 cells, of male human embryonic lung cells (HEL-M) and of Detroit-550 cells (human foreskin) has been obtained with BαP, 4NQO, MNNG, and occasionally with DMBA. Such cultures possessed increased cell density, greater doubling potential, losses of contact inhibition, increased cloning efficiency and ability to grow under or in soft agar. These effects were enhanced by the addition of 17 β estradiol or cortisone acetate. Transformation of Detroit-550 cells by SV_{40} was enhanced by pretreatment with BαP, MNNG, 4NQO and Acetoxy-AAF but not by MCA or DMBA.

Significance to Biomedical Research and the Program of the Institute: Although non-human mammalian cells have been malignantly transformed in vitro with known chemical carcinogens, there is no report of transformation of human cells by chemical carcinogens. It is of major importance to determine the conditions necessary to achieve neoplastic transformation of human cells by chemical carcinogens in vitro. An understanding of the process by which these carcinogens at least initiate the transformation process can be obtained by analyzing their ability to promote viral transformation of human cells.

Proposed Course: Since the present evidence indicates that certain carcinogens apparently at least initiate transformation (morphologic) of human cells, the major emphasis will be to augment or potentiate these changes. For example, Detroit-550 cells will be pretreated with MMS with and without hormones or DNA repair inhibitors prior to carcinogen treatment. Mixed-function oxidases will be added or induced in Detroit-550 cells prior to addition of polycyclic carcinogens. All transformants will be tested for their ability to form tumors in immunologic deficient hamsters or mice. Analysis of the enhancement and/or inhibition of SV_{40} transformation of human cells by carcinogens will be completed and similar studies with FSV initiated.

Date Contract Initiated: December 20, 1971

Current Annual Level: $104,982

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NIH-NCI-E-43277)

Title: Isolation and Chemical Characterization of Soluble Human Tumor (CEA) Specific Antigens

Contractor's Project Directors: Dr. Ralph A. Reisfeld
Dr. Gary S. David

Project Officer (NCI): Dr. Tibor Borsos

Objectives: The aim of this contract is to obtain human tumor specific antigens in a highly purified and chemically well-characterized form. This includes the development and application of new, large-scale purification methods and the thorough chemical and biological characterization of carcinoembryonic antigens (CEA) of colon cancer.

Major Findings: Earlier investigations supported by this contract resulted in the development of a solid-state enzymatic method of iodination of proteins, which has been used extensively in the iodination of CEA for radioimmunoassay (RIA) as well as trace-labeling of other materials. Further work has shown that this Sepharose-bound lactoperoxidase is capable of functioning under remarkably adverse conditions, such as in the presence of 10M urea or 0.1% sodium dodecyl sulphate (SDS). It has also been found that this insoluble enzyme is capable of incorporating radioiodine into extremely dilute proteins (microgram per ml range) in the presence of SDS. This capability, in conjunction with immunoadsorbent technology discussed below, is currently being investigated as a means of evaluating either antisera or antigens (specifically, CEA or CEA-like antigens) available only in minute quantities in sera or tissue extracts.

Antisera to CEA have been produced in goats by the injection of purified CEA and in rabbits by immunization with whole tumor cells. In general, tissues expected to contain CEA (i.e., gastrointestinal carcinoma tissue) yield antisera which are capable of binding ^{125}I-CEA, while CEA-negative cultured cells or normal tissue do not. In addition, it has been possible to produce antiserum to CEA in a monkey (macaque) by the injection of whole tumor tissue. The antibody formed, which was not of the IgG class is currently undergoing further investigation to determine its nature and specificity. A second monkey, which received injections of normal colonic tissue, failed to produce antibodies to CEA.

Rabbits were injected with Sepharose-based anti-CEA immunoadsorbents, which had been exposed to either CEA positive serum from a colonic carcinoma patient (metastatic) or to anti-CEA-reactive material from the serum of a normal individual, resulting in the removal of approximately 1-2 μg of anti-CEA-reactive material from each serum sample. Those rabbits which had received the CEA-exposed immunoadsorbent developed significant titers of anti-CEA antibody, while those receiving the normal serum-exposed immunoadsorbent did not. Further evaluations of the antisera and of this technique are being conducted.

The lectin concanavalin-A (Con-A) was found capable of binding CEA. CEA can be subfractionated into three categories using Sepharose-bound Con-A: approximately 20% of CEA from a tumor extract does not bind (or interacts very weakly) with Con-A Sepharose, while the remaining portion binds strongly. Of the bound fraction, about 80% can be eluted with 0.1M α-methyl-D-mannoside, while the remaining fraction cannot be eluted with this glycoside. Preliminary experiments suggest that Sepharose-bound Con-A may be valuable in the isolation of CEA from tumor extracts.

Other Sepharose-bound lectins tested to date, i.e., soybean agglutinin (N-Ac-galactosamine-specific), wheat germ lectin (N-Ac-glucosamine-specific) and MP-lectin (galactosamine-specific) interact only weakly, if at all, with CEA. On the other hand, each of these lectins is capable of binding four blood group substances tested--A, B, H and Le°. None of these glycoproteins was found to bind to Con-A-Sepharose. These data strongly suggest that a combination of Sepharose-bound lectins, probably in conjunction with molecular sieve chromatography and/or preparative electrophoresis, may provide a gentle and effective method of isolating and/or subfractionating CEA. It should thus be possible to avoid the harsh perchloric acid or lithium diiodosolacylate methods currently employed in CEA isolation procedure.

The binding of CEA by Con-A-Sepharose has yielded a technique useful in the radioimmunoassay procedure. Heavily radioiodinated CEA deteriorates rapidly upon storage. It has been possible to store this ^{125}I-CEA bound to Sepharose, then wash the beads and elute the ^{125}I-CEA with α-methyl-D-mannoside when needed. Radioiodinated, non-antigenic degradation products are released from the beads and removed during the washing procedure. Eluted ^{125}I-CEA binds to CEA-specific antibody to the extent of 90-100% even after 2 1/2 months storage.

The concentrations of Con-A and antibodies bound to Sepharose are quite important in determining optimal conditions for the use of these adsorbents. It has been found that the concentration of CNBr used to activate the Sepharose is important in determining the effectiveness of an anti-CEA immunoadsorbent used for radioimmunoassay purposes. Optimal conditions for developing immunoadsorbents for use in the RIA as well as for preparative use are currently being investigated.

Significance to Biomedical Research and the Program of the Institute: This contract would permit the immunochemical and chemical characterization of this antigen of human tumors and would contribute to the understanding of the development and possible etiology of this cancer. In addition, highly purified antigen of this tumor would greatly facilitate the development of refined second generation tools for the detection of human colon cancer.

Furthermore, technology developed here will provide new and valuable methods of isolating tumor antigens and of evaluating antisera and antigens related to other tumor systems.

Proposed Course: Highly purified CEA will be isolated from colonic adenocarcinomata and from sera and ascitic fluid from tumor-bearing patients obtained from the University of California at San Diego (NIH-NCI-E-72-3258)

and from the Mallory Institute of Pathology Foundation (NIH-NCI-E-71-2276). Procedures for isolation of CEA will be investigated, involving the use of Sepharose-bound lectins, immunoadsorbents and chromatographic and electrophoretic procedures. Attempts will be made to isolate CEA on a preparative scale as well as on an analytical scale for the purpose of comparing anti-CEA reactive material from a variety of sources. Antisera to CEA will be produced in a number of ways in an attempt to develop a battery of highly specific immunological reagents which would provide an unambiguous diagnostic tool.

Date Contract Initiated: December 1, 1971

Current Annual Level: $75,735

SORVALL, IVAN, INC. (NIH-NIAID-A1-32513)

Title: Fractionation of BCG Cell Walls: A Search for Chemically-Defined Products with Immunologic Anti-Cancer Activity

Contractor's Project Director: Dr. Edgar Ribi

Project Officer (NCI): Dr. Berton Zbar

Objectives: To fractionate cell walls from virulent, avirulent, and attenuated strains of mycobacteria to isolate, purify, and identify substances having tumor suppressive properties.

Major Findings: Cell walls from virulent Mycobacterium tuberculosis, M. bovis, and atypical M. kansasii were at least as effective in regressing established dermal tumors (transplantable line-10 hepatocarcinoma) in syngeneic guinea pigs and in preventing the growth of lymph node metastases as were cell walls from attenuated M. bovis, strain BCG. Cell walls from these organisms had in common the compound designated P3, which is a trehalose mycolate. Cell walls from avirulent M. smegmatis which do not contain P3 were not effective but were rendered tumor suppressive when combined with P3 that had been isolated from M. tuberculosis. Similarly, the water-soluble mycobacterial adjuvant and antitumor factor (MAAF) described by Hiu was capable of regressing tumors only after being admixed with P3. Hence, P3, which itself does not possess tumor regressive activity, plays an essential role in this activity shown by viable BCG vaccines and mycobacterial cell wall preparations. P3 was also found to be an equally essential ingredient of the products eliciting enhancement of resistance in laboratory animals against airborne infection with virulent tubercle bacilli. P3 consists of trehalose esterified with several different mycolic acids and because these enormous C90 acids are difficult to obtain as single, homogeneous entities, their structural details are largely unknown. With the aid of low-pressure elution and centrifugal microparticulate gel chromatography, these mycolic acids, liberated from P3 by saponification, were separated as methyl esters into four distinct entities. The mass spectra of two of them resembled that of a known mycolic acid (α-mycolic acid); the structural details of the other two are not know.

Significance to Biomedical Research and the Program of the Institute: Although the antitumor effectiveness of BCG has been convincingly demonstrated in laboratory animals, it has been difficult to obtain unequivocal evidence that treatment with BCG prolongs the survival time of patients having malignant disease. The major difficulty has been that the disease in patients so treated has been much further advanced than the disease in animals where the treatment succeeded. Since mycobacterial cell walls could be used in lieu of the viable BCG cells, they are an appropriate source for obtaining the immunotherapeutical entities. Also, from a theoretical point of view such studies will aid in the elucidation of the molecular basis of the immunopotentiating action of BCG or mycobacteria in general.

Mycobacterial lipids containing mycolic acids play an important role in stimulating cellular immunity and tumor regressive activity and now, for the first time, the use of chromatographic techniques developed here has made it possible to separate these high carbon number acids from natural mixtures.

Proposed Course: Studies are in progress to determine 1) the exact structure of P3 in order that it might be synthesized, 2) the minimal chemical and structural features of the cell wall required in combination with P3 to bring about regression of tumors and subsequently to establish systemic tumor immunity, and 3) whether delayed hypersensitivity to the tubercle bacillus itself, which is vigorously enhanced by the action of P3 on tuberculoprotein or peptides, is required for tumor rejection. Tuberculin will be substituted for the tumor specific antigen and thereby channel the major immune response toward it. The noxious side effects of tuberculin hypersensitivity would be eliminated and, hopefully, the cell-mediated immunity toward tumor specific antigen would be enhanced by reducing antigenic competition.

This project also will provide a continuing source of mycobacterial cell walls and fractions derived from them for distribution to other approved investigators who request them.

This contract was completed and a new contract, or extension of this one, is being negotiated with Hamilton Biochemical Research Laboratory; it will be the same team; only the name of the company has been changed.

Date Contract Initiated: January 9, 1973

Current Annual Level: $39,676

TEMPLE UNIVERSITY (NO1-CP-33200)

Title: Induction of Malignant Melanoma in Guinea Pigs

Contractor's Project Director: Dr. Wallace H. Clark, Jr.

Project Officer (NCI): Dr. Herbert Rapp

Objectives: To develop a model in a guinea pig which is analogous to one form of human cutaneous malignant melanoma; to determine whether or not this model can be altered by immunopotentiation with BCG or altered by immunosuppression with anti-lymphocyte globulin.

Major Findings: In an attempt to develop a model for malignant melanoma in the guinea pig, animals are being treated with DMBA, DMBA plus ultraviolet light, and DMBA plus ultraviolet light and psoralens.

Animals treated with DMBA show significant changes. At the sites of painting there is uniform hyperpigmentation, and within this hyperpigmentation there are four different kinds of lesions. The first of these lesions is a slightly elevated blue black nodule varying from less than 1 mm. to about 6-7 mm. in diameter. These nodules show a slight increase in the number of intra-epidermal melanocytes. The nodule is formed by a lesion in the dermis which consists of melanophages and melanocytes. Some melanocytes are slightly atypical but one cannot interpret this lesion as malignant melanoma. There is also a moderate increase in the number of intra-epidermal melanocytes throughout the painted area with striking prominence of dendrites containing large quantities of melanin.

At about 52 weeks small brown pigmented lesions developed within the painted areas. These may represent the early, true hyperplasia of melanocytes that possibly could be the beginning of the centrifugal growth phase of melanoma.

By the time 5 weeks has gone by, there is a striking increase in melanin synthesis in all painted animals suggesting that DMBA is a stimulator of melanin synthesis. In most human and animal systems, the only known mechanism for stimulating pigment synthesis is light. This carcinogen clearly induces pigment synthesis. It also apparently is not associated with pigment transfer from melanocytes to keratinocytes in any significant quantity. The induced synthesized pigment largely remains in the melanocytic system. This is in contrast to the effect of light. This phenomenon, if correct, may be of vital importance in studying melanogenesis and may be of importance in carcinogenesis, but this awaits the full development of this model.

It was also observed that the younger the animal is when the experiment starts the more likely these changes are to develop, and animals receiving DMBA in double dosage have lesions appearing 8-10 weeks earlier than animals receiving a single application of DMBA.

All of the animals that are beyond 52 weeks have apparently developed definite keratinocytic lesions. These keratinocytic lesions may easily be interpreted as squamous cell carcinoma in-situ with a definite decrease in cellular cohesiveness. This squamous cell carcinoma is much closer to human squamous cell carcinoma than are lesions in mice.

Electron microscope studies revealed that there is a great increase in the amount of pigment synthesis with apparent block in pigment transfer due to DMBA. There are definite melanocytes in the dermis in the small blue nodules and an increased number of melanocytes. Abnormal melanin granules are being formed in some of the melanocytes.

At the fine structural level in the keratinocytic system, one of the earliest changes is abnormalities in the cell membrane with complex enfoldings and a decreased number of cellular attachments.

Significance to Biomedical Research and the Program of the Institute: The major goal of this project is to produce a tumor which is similar to human cutaneous malignant melanoma, with a biphasic growth pattern progressing to malignancy. If primary tumors with significant potential for biological malignancy evolve, the guinea pig model can be used for both immunotherapy and chemotherapy studies which have the possibility of direct applicability to man. An attempt to manipulate the evolution of a primary tumor that will progress to full biological malignancy through immunological means may block tumor progression and may illuminate the nature and behavior of malignant melanoma in man.

Proposed Course:

(1) To continue these experiments until it can definitely be stated that a model of malignant melanoma analogous to man is or is not produced and to delineate the exact analogies in the exact places where this model differs; (2) To push both light and psoralens to the point where the time course and the yield can hopefully be accelerated; (3) After a model has been developed, to attempt to alter the model with immunopotentiation and immunosuppression; and (4) To clearly delineate this as a model for squamous cell carcinoma of the skin in man.

Date Contract Initiated: October 1, 1972

Current Annual Level: $71,000

TEXAS, UNIVERSITY OF (at San Antonio) (NO1-CP-23210)

Title: Development of *In Vitro* Methods for the Detection of Cell-Mediated Immunologic Reactivity to Chemical Carcinogens

Contractor's Project Director: Dr. Daniel E. Thor

Project Officer (NCI): Dr. Herbert Rapp

Objectives: To determine the feasibility of devising *in vitro* immunologic tests for the detection of prior exposure to known chemical carcinogens.

Major Findings: Maximum guinea pig sensitization to three carcinogens, DMBA, 3-MCA, and BP, has been achieved using a modified split-adjuvant technique. Utilization of recrystallized carcinogens for skin testing has allowed greater specificity of skin test reactions. Collaborative studies have been initiated to evaluate specific antibody to carcinogens that also reacts with each of our specific carcinogen-carrier complexes.

Presently available data shows that (1) direct addition of carcinogens in a variety of solvents are either cytotoxic to non-sensitized and sensitized lymphocytes and peritoneal exudate cells (PECs) or they can not, as haptens trigger a cell-mediated immune response in vitro, (2) carcinogen-albumin carrier complexes are water soluble and can inhibit sensitized guinea pig PECs in a migration inhibition assay (MIF) for CMI, and (3) the carcinogen-carrier complexes are not reactive or toxic with normal, nonsensitive guinea pig PECs. A microtechnique for the assay of macrophage migration inhibition and migration inhibition factor (MIF) has been devised to screen carcinogen-carrier complexes. Both direct and indirect MIF assays have been positive in highly carcinogen-sensitive guinea pigs utilizing this agarose droplet technique. The in vitro preparation of human carrier-carcinogen complexes have been initiated.

Significance to Biomedical Research and the Program of the Institute: If successful, these studies will make it possible to determine whether human individuals have been exposed to a variety of agents known to be carcinogenic in animals and suspected of being carcinogenic in man. The development of an immunogenic carcinogen-carrier complex with specificity might also be very useful in surveillance studies. Significant differences among various populations in their immunologic reactivity to known carcinogens might make it possible to correlate the degree of reactivity with the subsequent development of cancer. Correlations of this kind might provide the impetus to remove the agents from the environment.

Proposed Course: Utilizing guinea pigs highly sensitized to chemical carcinogens, experiments are in progress to evaluate specificity in vitro and in vivo. Although MIF data appears to differentiate sensitized and nonsensitized animals, blast transformation may still be feasible if high background nucleotide incorporation can be minimized or eliminated. Major efforts will be made to improve mole ratios of carcinogen-carrier complexes and evaluate the most efficient macromolecular carrier.

Date Contract Initiated: February 16, 1972

Current Annual Level: $76,094

TRUDEAU INSTITUTE, INC. (NO1-CP-23221)

Title: Tumor Inhibition by Mycobacteria: Standardization of Mycobacterial Preparations

Contractor's Project Director: Dr. George B. Mackaness

Project Officer (NCI): Dr. Berton Zbar

Objectives: 1) To compare the capability of different mycobacteria to a) inhibit tumor cell growth or b) augment the development of specific tumor immunity; 2) To compare the influence of different methods of bacterial culture and preservation on capability of mycobacteria to inhibit tumor cell growth; 3) To provide NCI with 800 vials (1 ml frozen each) of BCG (or other

mycobacteria); and 4) To compare different mycobacteria for safety of administration.

Major Findings: Tumor suppressive activity of various BCG strains has been studied both in guinea pigs and mice.

(1) Guinea Pig Assay: Strain 2 guinea pigs were used for comparing the tumor suppressive activity of different strains and preparations of BCG in terms of their capacity to cause regression when injected into Line 10 tumors.

The most interesting strains of BCG are the three used most frequently for immunotherapeutic purposes in man. A comparison was, therefore, made of these three strains (Pasteur, Tice, and Glaxo) using fresh-frozen organisms with known viable counts. The results show that the Pasteur strain outperformed the other two and was much superior to the Glaxo Strain, which scarcely influenced tumor growth at all.

It was further found that commercial lyophilized BCG is less potent than BCG grown in dispersed culture and preserved by freezing at -70°C. The admixing of lyophilized BCG with the fresh-frozen product interfered with the tumor-suppressive activity of the latter. It is thought that the large quantities of antigenically active, but immunotherapeutically inert, material in freeze-dried vaccines may be responsible for this effect.

(2) Mouse Assay: BCG (Pasteur or Phipps) exerts a modulating influence on the systemic immune response to the poorly immunogenic murine mastocytoma P815 (MA), when irradiated MA are injected into sites prepared by a prior injection of BCG. The degree of immunopotentiation, monitored against live MA located at a distant site, depends critically on timing. The first sign of tumor inhibition occurred 7 days after the tumor-specific immunogenic stimulus was introduced and maximum antitumor immunity was achieved when irradiated tumor cells were injected 10 days after the priming dose of BCG. The tumor-suppressive effect was almost log linearly related to BCG dose, but raising the dose of irradiated tumor cells beyond a certain optimum caused less suppression and lower survival rates.

Significance to Biomedical Research and the Program of the Institute: Although its mode of action is not fully understood, BCG continues to be used for suppression of tumor growth both in experimental animals and man. It is clear that different substrains of BCG vary markedly in their immunopotentiating activity, and that further investigation is necessary to insure selection of the strain which will provide maximal anti-tumor effect. Even more striking is the significantly greater tumor suppressive activity of fresh-frozen BCG compared to commercial lyophilized preparations of the same vaccine strain. It is thought that large quantities of soluble, antigenically active, but immunotherapeutically inert material in the freeze-dried vaccine may be responsible for this effect. If studies are successful, it should be possible to develop a fresh grown BCG of high viability which may be stored frozen or lyophilized and which is not accompanied by the immunosuppressive effects currently associated with commercially available freeze dried products.

Proposed Course: To prepare BCG in a form that gives greater and more reliable tumor suppressive effects through more careful selection of genotype, and by use of procedures which enhance vaccine activity by minimizing (or eliminating) undesirable immunologic effects such as those caused by antigens released from dead or disrupted bacilli. It is hoped that the mode of action of BCG may be precisely established so that full advantage may be taken of its immunotherapeutic effect without imposing restrictions on dose or route of administration.

Date Contract Initiated: February 24, 1972

Current Annual Level: $201,379

UTAH, UNIVERSITY OF (NO1-CP-33263)

Title: Carcinogenesis Bioassay Resource for Determining the Effect of Chronic Immunosuppression on Physical and Chemical Carcinogenesis

Contractor's Project Directors: Dr. E. J. Eichwald
Dr. M. L. Kripke

Project Officer: Dr. Curtis C. Harris

Objectives: (1) To determine the effects of chronic treatment with cyclophosphamide, methotrexate, azathioprine, prednisolone, azathioprine + prednisolone, and ALS on benzpyrene and ultraviolet (UV) skin carcinogenesis in mice, and (2) To investigate immunological aspects of UV carcinogenesis in mice.

Major Findings: (1) Appropriate doses of benzpyrene and UV light to use in studies combining treatment with these carcinogens and immunosuppressive drugs have been defined. (2) Dose and regimen for each of the immunosuppressive agents which permit chronic drug administration without excessive toxicity have been determined. (3) The effects of the immunosuppressive drugs on skin allograft rejection have been investigated. (4) It has been found that tumors induced by UV light exhibit an unusually high degree of antigenicity, and that they are not detectably cross-reactive when tested by transplantation techniques.

Significance to Biomedical Research and the Program of the Institute: This study is directed toward the following questions: Is the current trend toward increasing clinical use of immunosuppressive drugs likely to elevate the incidence of human malignant disease? Can the effects of these drugs on carcinogenesis be directly attributed to their immunosuppressive activity? Attempts are being made to provide information related to these questions through the use of carefully defined animal model systems. In addition, findings on the antigenicity of UV induced tumors provide a unique opportunity to investigate the mechanism which enables highly antigenic tumors to escape immunologic destruction in the primary host.

Proposed Course: (1) Proceed with studies combining treatment with carcinogens and immunosuppressive agents, (2) Determine the immunosuppressive activity of the doses of drugs employed in the combination experiments in a variety of immunological test systems, and (3) Study immunological parameters of UV carcinogenesis.

Date Contract Initiated: May 7, 1971

Current Annual Level: $215, 490

SUMMARY REPORT

CARCINOGEN METABOLISM AND TOXICOLOGY SEGMENT

July 1, 1973 through June 30, 1974

The Carcinogen Metabolism and Toxicology Segment has as one of its goals the identification of those components of complex systems which function as environmental carcinogens and the development of analytical methodology for their detection and quantitation. Metabolic and toxicologic studies of environmental carcinogens in selected animal systems are conducted to provide additional knowledge on the metabolic pathways involved in the carcinogenic process. Such studies may also furnish an explanation for the varying responses of different animal species and man to chemical carcinogens. Mechanisms of chemical carcinogenesis in selected animal systems with defined characteristics are being conducted.

Nitrosamines program

One of the primary concerns of the Segment is the development of a program of research on N-nitrosamines and their possible involvement in so-called spontaneous cancer in humans. In this area several contracts are being supported by the Segment. Massachusetts Institute of Technology (NCI-33315) is directed primarily at the possible formation of nitrosamines in foodstuffs during preparation and storage or from endogenous materials consumed with the diet. This project has shown that the food additive, sodium nitrite, can react with other food constituents under a variety of conditions of temperature and pH to form some nitrosamines. The kinetics of some of these reactions which may occur even in the frozen state are being investigated. Foods and beverages from regions high in specific cancer incidence are also being investigated to determine whether nitrosamines may be responsible.

In addition, the formation of nitrite in saliva from endogenous and dietary nitrate by the bacteria present in the dental plaque is being investigated. An attempt is being made to identify the microorganisms responsible and to determine under what conditions the reduction of nitrate occurs. Since nitrate is a constituent of many vegetable foods, the implications of its reduction to nitrite with further reaction with dietary amines are obvious. Investigations are also being made of naturally occurring compounds such as ascorbic acid and gallic acid which inhibit the formation of nitrosamines from nitrite. At the AEC Oak Ridge National Laboratory a related study is being done with emphasis on the formation of nitrosamines from tertiary amine drugs which are rather commonly used in this country. The reactions by which tertiary amines dealkylate in the presence of nitrite to form dialkylnitrosamines are being studied as well as the conditions which favor or inhibit these reactions. In this project the metabolism and carcinogenic activities of the nitrosamines thus formed are also being investigated. The Segment is also responsible for that portion of the Eppley Institute contract (University of Nebraska) dealing with nitrosamines. In this project, the synthesis of various model nitroso compounds and the testing of some of these compounds for carcinogenic activity

are being pursued. Further studies on the chemistry and kinetics of nitrosation reactions and the effects of blocking agents are also conducted at the Eppley Institute. Previously there has been little information available on the levels of certain ureas and guanidines which occur naturally in foods or as endogenous materials in mammalian organisms. Since many nitrosoureas are readily formed *in vitro*, it is of importance to determine what levels of the precursor ureas, guanidines and secondary amines occur in the environment. The analytical methods for detection of such compounds are not very well defined and studies on this area are being pursued. The carcinogenicity of various model nitrosourea compounds and possible metabolites such as the beta-oxidized nitrosamines are being investigated. Investigators at the Eppley Institute are also testing the effect of ascorbic acid on inhibition of nitrosation of secondary amines. The effects of the enzyme inducer pregnenolone 16α-carbonitrile on nitrosamine metabolism and carcinogenesis are also studied.

Mechanistic and metabolic studies

As part of its efforts on the metabolism of carcinogens, the Segment is supporting two projects at the Massachusetts Institute of Technology which bear on this topic. One of these (Contract NCI 4-3265) is involved with aflatoxin and other mycotoxins which have been identified on samples of foodstuffs from Southeast Asia. The metabolism of aflatoxin *in vivo*, its effects on cell systems, and its *in vitro* metabolism in tissues from various species including man are being studied. Several mycotoxins which have been identified from other foodstuffs from Thailand have an extremely powerful physiologic action. Their possible carcinogenic activity is being determined in long term studies. Another project (Contract NCI-33238) investigates the effects of lipotropes such as choline and methionine and their effects in chemical carcinogenesis. In view of the high incidence of colon cancer in much of the western world, these studies emphasize largely the effects of lipotropes on colon carcinogens such as dimethylhydrazine in rats and mice. Although the lipotropes have an effect on some types of chemical carcinogens, a definitive mechanism for their action has not been elucidated. A relatively short term bioassay, based on lung adenoma induction in strain A mice, is used to test various alkylating agents, drugs, food additives, alkyl halides and other chemicals (University of California, San Diego, NCI-33232). This technique, within its limitations, furnishes a relatively quick method for screening of presumed carcinogens and also allows studies on the combined effects of various non-carcinogenic viruses and chemical carcinogens, and inhibitory effects in chemical carcinogenesis. The Segment is also supporting a project (Temple University) on the possible occurrence of carcinogenic mycotoxins in dermatophytes which are commonly found in humans. In many cases these organisms are found in overwhelming numbers in cancer patients. Therefore studies are being done on the possible carcinogenic and co-carcinogenic effects of some of the fractions isolated from these fungal cultures.

Mutagenesis prescreening program

An important facet of the Segment activities is involved with the development and validation of mutagenesis test methods for prescreening compounds for possible carcinogenic activity. There is considerable evidence that many compounds which are mutagenic are also carcinogens and that many carcinogens are also mutagenic, especially in an activated form. Several contracts are being carried presently to determine the correlation between the carcinogenicity and mutagenicity of 85 compounds (known carcinogens of various structural types and non-carcinogenic analogs) in various bacterial systems both with and without an activation system (Stanford Research Institute, Columbia University). These same compounds are also used in a mouse lymphoma cell system (A. D. Little, Inc.) and in a DNA repair system (Stanford Research Institute) with a view to defining the effectiveness and reliability of short-term prescreening methods. Some of the preliminary results from the bacterial mutagenicity studies are quite promising, in that there is a good correlation between carcinogenicity and the mutagenic activity.

CONTRACT NARRATIVES

CARCINOGEN METABOLISM AND TOXICOLOGY SEGMENT

July 1, 1973 through June 30, 1974

AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratories) (72-204)

Title: Role of Nitrosamines in Carcinogenesis

Contractor's Project Director: Dr. William Lijinsky

Project Officer (NCI): Dr. John Cooper

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis (AEC-NCI Interagency Agreement) (NCI-40013)

ARTHUR D. LITTLE, INC. (NO1-CP-33392)

Title: Use of Lymphoma Cells *in Vitro* and in Host-Mediated Bioassays as a Potential Prescreen for Chemical Carcinogens

Contractor's Project Director: Dr. Jerry Carter

Project Officer (NCI): Dr. Lionel Poirier

Objectives: The objective of this project is to determine if unique phenotypic expressions in mouse lymphoma cells can be used as a means to identify chemicals with carcinogenic potential. If so, then this system will eventually be one in a battery of mutagenicity assays used as prescreens of evironmental chemicals for carcinogenicity.

Major Findings: *In Vitro*: Twenty-eight compounds have been tested for toxicity to L5178Y lymphoma cells *in vitro*. In the mutagenicity prescreening series, ten of these compounds are tentatively considered mutagenic in at least one locus. Two are mutagenic at two different loci. Three selective agents, cytosine arabinoside, methotrexate and thymidine are used as genetic markers. The *in vitro* system using microsome preparations for metabolic activation of compounds is in the initial stages. Results from these studies are not complete.

In Vivo: Twelve compounds tested in the host-mediated assay system are tentatively mutagenic in at least one locus. Four are mutagenic at two different loci. There is a positive correlation of results in the two systems for some compounds. Mutagenic activity is tentatively positive at the same locus *in vitro* and *in vivo* for six of the compounds tested.

Significance to Biomedical Research and the Program of the Institute: A completely successful termination of this contract would be of major importance to the Natonal Cancer Institute. One of the main aims of the National Cancer Institute is the detection and elimination of potential carcinogens from the human environment. The development of prescreens with a high degree of correspondence between mutagenesis and carcinogenesis would greatly simplify that task. This contract is one attempt to obtain such a prescreen.

Proposed Course: To continue with the studies as planned and complete the prescreening tests on the remaining compounds.

Date Contract Initiated: June 19, 1973

Current Annual Level: $109,500

CALIFORNIA, UNIVERSITY OF (at San Diego) (NO1-CP-33232)

Title: Pulmonary Tumors in Mice for Carcinogenic and Cocarcinogenic Bioassay

Contractor's Project Directors: Dr. Michael B. Shimkin
Dr. A. J. Kniazeff

Project Officer (NCI): Dr. Elizabeth K. Weisburger

Objectives: The objectives develop along two lines:

(1) Bioassay - Testing of environmental chemicals for carcinogenic activity using the strain A mouse pulmonary-tumor-induction-technique developed by Shimkin and Andervont in 1940. A variety of chemicals have been proposed for testing (food additives from the GRAS list, metals, chemotherapeutic drugs, fumigants, silylating agents, and related compounds).

(2) Co-carcinogenicity Studies - The interaction of non-oncogenic viruses and chemicals on the induction of pulmonary tumors in strain A mice is being investigated. Mice are exposed to virus by the inhalation route. After establishment of infection, the mice are treated with a chemical carcinogen (3-methylcholanthrene or urethan). The nature of possible virus-chemical interaction is being evaluated by quantitation of the developed lung adenomas.

Major Findings: (1) Bioassay - During the past year, the strain A mouse pulmonary tumor bioassay has been applied to the carcinogenicity testing of 22 food additives, 16 organohalide chemicals, 10 silylating agents, and one tobacco smoke condensate from NCI Code 53 cigarettes. The organohalide chemicals are mostly industrial intermediates. The silylating agents are used for gas-liquid chromatography. The tumor response of chemically treated mice was compared to that of the appropriate vehicle controls with the following results: (a) food additives - On first test, alpha-methylcinnamic aldehyde, isobutyl salicylate and phenylethylphenylacetate were carcinogenic. Repeat tests for these chemicals are underway. All other food additives were negative at the dose levels employed. (b) Organohalide chemicals - Carcinogenicity of

the organohalides was found to be related to two factors; stability and structure. The more unstable iodides and bromides were more active than the chlorides. Chemicals with primary structures were generally inactive, whereas those with secondary and tertiary structures were active. (c) Silylating agents - Tests on carcinogenicity of the 10 silylating agents have not been completed. (d) Cigarette smoke condensate - At the dose levels employed, NCI Code 53 cigarette smoke condensate was negative for pulmonary tumor induction in strain A mice.

(2) Co-carcinogenicity Studies - (a) Virus-chemical interaction in the development of pulmonary tumors in strain A mice - Treatment of strain A mice with the Moloney strain of murine sarcoma virus causes a 30 to 50% reduction in 3-methylcholanthrene (MCA) and urethan-induced adenomagenesis. Animals received virus 14 days prior to treatment with MCA or urethan. The viral inhibitory effect was not related to the virus dose but was related to the dose of chemical; i.e., the reduction in tumor incidence among viral-chemically treated groups receiving the lower dose of chemical was greater than in those receiving the higher dose.

Co-carcinogenesis studies are currently underway using non-oncogenic murine viruses; e.g., Reovirus, adenovirus, and chemical carcinogens. Data from these studies are not available as yet.

Significance to Biomedical Research and the Program of the Institute: This project will integrate into the activities of the Carcinogenesis Program by attempting to develop a rapid and reliable bioassay of chemicals for carcinogenic activity. Research in this area is of high priority due to the vast expense and time required to screen chemicals by the presently used animal bioassays. This research project will also provide timely extension of the existing bioassay capabilities.

Proposed Course: To continue work in the areas outlined above. The effect of certain inhibitors of chemical carcinogeneis will be evaluated in the bioassay system. Compounds already known to be positive or negative for carcinogenic activity will be retested in this system to determine the degree to which the results parallel those of conventional bioassays of long-term duration.

Date Contract Initiated: June 22, 1970

Current Annual Level: $147,558

COLUMBIA UNIVERSITY (NO1-CP-33395)

Title: *In Vitro* and Host-Mediated Microbial Mutagenicity as a Prescreeen for *Chemical* Carcinogens

Contractor's Project Director: Dr. Herbert Rosenkranz

Project Officer (NCI): Dr. Lionel Poirier

Objectives: The objective of this project is to determine if mutagenicity in bacteria can be used as a tool to identify carcinogenic potential. If so, then this system will eventually be one in a battery of mutagenicity assays used as prescreens of environmental chemicals for carcinogenicity.

Major Findings: Eighty-five chemicals comprising a series of known carcinogens are in the process of being tested for (a) ability to induce mutations in Salmonella typhimurium using a host-mediated assay (mice); (b) ability to induce mutations in S. typhimurium in vitro in the presence and absence of a cell-free activation mixture and (c) capacity to preferentially inhibit the growth of DNA polymerase-deficient Escherichia coli (E. coli pol A_1^-) in the presence and absence of a cell-free activation mixture.

The results obtained thus far indicate the following: (1) There is a good correlation between the results of the in vitro tests (in the absence of metabolic activation) and the ability of a substance to act as a direct carcinogen. (2) Substances that require metabolic activation prior to exerting their oncogenic potential are not active in the in vitro systems in the absence of the cell-free activation mixtures. In the presence of such activation mixtures only certain of these proximate carcinogens were activated. In general, aromatic hydrocarbons remained inactive. (3) Substances that gave direct positive tests in the in vitro assays frequently gave negative results upon the addition of the cell-free activation mixture. This was caused by the reaction of these chemicals with components of the mixture; they thus became unavailable to the test microorganisms. Similarly, some proximate carcinogens were converted to the active intermediates upon addition of the activation mixture but under the normal conditions of the assay, the intermediates reacted rapidly with components of the mixture and were thus unavailable to the bacteria (e.g., dimethylnitrosamine). (4) Similarly, a number of direct activating carcinogens yielded negative results in the host-mediated assay; presumably they did not reach the test bacteria because of their reactivity. (5) Only substances that could be activated metabolically in vitro gave unequivocal positive results in the host-mediated assay. A number of chemicals gave borderline results in this assay; these were difficult to interpret. (6) As routinely used, the E. coli pol A_1^- and S. typhimurium in vitro assays gave essentially similar results. A number of agents gave positive tests only in the E. coli pol A_1^- but not in the S. typhimurium systems as used routinely. It was shown, however, that some of these were mutagenic for S. typhimurium when more elaborate procedures were followed. (7) A modified "host-mediated" assay was developed. In this procedure the urine of rats injected with proximate carcinogens is assayed in the in vitro systems. This method appears promising for evaluating the urines of persons receiving medication or exposed to environmental agents.

Significance to Biomedical Research and the Program of the Institute: A completely successful termination of this contract would be of major importance to the National Cancer Institute. One of the main aims of the National Cancer Institute is the detection and elimination of potential carcinogens from the human environment. The development of prescreens with a high degree of correspondence between mutagenesis and carcinogenesis would greatly simplify that task. This contract is one attempt to obtain such a prescreen.

Proposed Course: Complete the testing of 85 carcinogens and non-carcinogens and analyze the results to determine the reliability of in vitro procedures in predicting carcinogenicity.

Date Contract Initiated: June 18, 1973

Current Annual Level: $65,135

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1-CP-33238)

Title: The Interactions between Diet and Chemical Carcinogenesis: A Bioassay System

Contractor's Project Director: Dr. Adrianne E. Rogers

Project Officer (NCI): Dr. Lionel Poirier

Objectives: (1) To determine whether comsumption of a diet marginally deficient in lipotropes, which serves as a model for the diet of alcoholic and other malnourished human populations, enhances cancer induction by chemicals in rats; (2) To confirm and extend previous observations of the antagonism between dietary methyl donors and chemical carcinogens; and (3) To develop diets for experimental animals to be used in testing procedures for chemical carcinogens and in evaluation of environmental factors related to human carcinoma.

Major Findings: Studies of four carcinogens, diethylnitrosamine (DEN), dibutylnitrosamine (DBN), dimethylhydrazine (DMH), and dimethylnitrosamine (DMN) are completed.

DEN, DBN AND DMH were more effective carcinogens for the liver (DEN, DBN), the esophagus (DEN) or the colon (DMH) in lipotrope-deficient rats than in normal rats. Tumor induction by DMN and its metabolism in vitro by liver slices were not affected by diet. Aflatoxin B_1 was a more effective carcinogen in the deficient rats, as we have found in earlier studies. In a preliminary study, N-2-flourenylacetamide induced hepato carcinoma earlier in deficient rats. Therefore, lipotrope deficiency enhances the susceptibility of liver, esophagus and/or colon to carcinogenesis by several classes of carcinogen. The effect has been found in 5 of 6 carcinogens tested and has been reported for 2 other compounds, ethionine and dimethylaminoazobenzene, by others making a total of 7 of 8 carcinogens tested having increased effect in lipotrope-deficient rats. The marginal deficiency used in these studies induces fatty liver but not cirrhosis, depressed hepatic drug metabolism, and supports normal weight gain.

Significance to Biomedical Research and the Program of the Institute: The findings obtained in these experiments are of considerable practical and theoretical importance. The demonstration that several classes of chemicals are more effectively carcinogenic for the liver and other organs in lipotrope-deficient rats than in normal rats is of significance for human disease and

the attempts of NCI to find ways to prevent human disease. First, the induction of hepatocarcinoma and other tumors in rats is a major assay for substances potentially carcinogenic for man. The enhanced sensitivity of test animals fed lipotrope-deficient diet can be used both to shorten the time required for assay and to investigate compounds whose carcinogenicity is of such low magnitude that the results are in dispute. Second, hepatocarcinoma and esophageal carcinoma in the U. S. are correlated with cirrhosis and alcoholism which are, in turn, correlated with malnutrition. In other countries, tumors of these 2 organs also are correlated with malnutrition. The basis for the association between hepatocarcinoma and cirrhosis appears, from the current studies, to be enhanced susceptibility of the nutritionally-damaged liver to environmental carcinogens rather than carcinogen exposure peculiar to alcoholics. Identification of other organs such as esophagus and colon in which abnormalities of nutrition enhance chemical carcinogenesis may explain and suggest remedies for the epidemiology of other tumors in people. Among such remedies may be alteration of diet to decrease the susceptibility of people to environmental carcinogens in addition to attempts to decrease exposure to the carcinogens. Examination of the role of methyl donors and related compounds in chemical carcinogenesis may yield information leading to preventive, retardative, or therapeutic measures against human cancer.

Proposed Course: There are 5 carcinogens now being studied, and 2 more will be studied this year to increase the number of classes of compounds and the number of target organs examined in assessing the extent of the effect of lipotrope deficiency on chemical carcinogenesis. Using DEN as a test compound, individual dietary factors in which the control and deficient diets differ will be examined for their contribution to the enhancement of carcinogenesis by lipotrope deficiency.

Date Contract Initiated: February 1, 1972

Current Annual Level: $99,034

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1-CP-33315)

Title: Environmental Occurrence of N-Nitroso Compounds

Contractor's Project Directors: Dr. Steven R. Tannenbaum
Dr. Michael C. Archer

Project Officers (NCI): Dr. Elizabeth Weisburger
Dr. Larry Keefer

Objectives: The objectives of this contract are (1) to develop analytical procedures for the isolation and detection of N-nitroso compounds in foods and beverages, (2) to examine foods and beverages for N-nitroso compounds and their precursors in cancer-rich areas, (3) to define conditions which favor the formation of nitrosamines and conditions which inhibit their formation, (4) to determine the toxicity of newly discovered N-nitroso compounds, and (5) to determine conditions favoring the formation of nitrite from its precursors in the oral cavity.

Major Findings: A new solvent-partitioning procedure for extraction of heterocyclic as well as dialkylnitrosamines from food samples has been developed. High-performance liquid chromatography has been introduced as a rapid clean-up procedure. These methods have been applied to the determination of nitrosopyrrolidine in bacon. New methods have been developed for the quantitative determination of nitrite. Analysis of food and beverages from cancer-rich areas have thus far failed to reveal significant levels of nitrosamines. The effect of ascorbic acid on the kinetics of nitrosation reactions has been shown to depend markedly on the presence of oxygen. Nitrite has consistently been found in human saliva at levels of 6 ± 6 ppm. Salivary nitrite contributes more to human exposure to nitrite than any other source. A major route for nitrite formation in saliva is microbial reduction of nitrate. Enrichment of ^{15}N-nitrogen in salivary nitrogen has been found to occur when individuals are administered ^{15}N-glycine.

Significance to Biomedical Research and the Program of the Institute: The carcinogenicity of large numbers of nitrosamines has been demonstrated in various tissues of many animal species. These compounds which can form in the environment could represent a health hazard and may be relevant to the etiology of human cancer. This project will aid in determining the total extent to which nitrosamines may be present in the environment and conditions which favor or retard their formation in foods and beverages during their processing, storage, and consumption.

Proposed Course: The project will continue in the areas outlined above. Analytical techniques will be developed for the nitrosoamino acids. Samples from geographical regions having a high incidence of cancer will be examined for nitrosamines and their precursors. New factors will be investigated which affect the rate of formation of nitrosamines. Using ^{15}N-labeled compounds, the precursors of nitrite in saliva will be identified and conditions determined which favor nitrite formation.

Date Contract Initiated: June 11, 1970

Current Annual Level: $105,832

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1-CP-43265)

Title: Toxicity and Carcinogenicity Associated with Fungal Growth on Foodstuffs

Contractor's Project Directors: Dr. Gerald Wogan
Dr. George Büchi

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: (1) To investigate the biochemical, toxic, and carcinogenic effects of aflatoxins, their metabolites and analogs; (2) To determine the metabolic fate of aflatoxins in various species; (3) To synthesize aflatoxin metabolites; and (3) To isolate, characterize and investigate the toxicity and carcinogenicity of other mycotoxins produced by molds isolated from foods

or food raw materials.

Major Findings: Studies on toxicity and carcinogenicity of P_1 are in progress. Metabolites of aflatoxin B_1 are being isolated from mouse urine for structure elucidation. Metabolism by microsomal preparations from livers of animal species and man is being studied in vitro. A new aflatoxin derivative, aflatoxin Q_1, has been isolated and identified as a major metabolite produced by primates, including man. Fermentation techniques are being established for the production of approximately 30 additional toxin-producing fungi isolated from Asian foodstuffs.

Significance to Biomedical Research and the Program of the Institute: Since mycotoxins have been suggested as being implicated in the etiology of human cancer, efforts to detect these compounds in the human environment should be useful in determination of causes of cancer and detection of the risk of selected populations. Elucidation of new mycotoxins produced by food spoilage fungi could lead to effective prevention, since this information could lead to preventive measures for control or removal from the food supply. This project is producing information on the total extent to which mycotoxins are present in the environment, particularly in selected geographic areas and populations that have elevated incidence of liver cancer of unknown etiology, thus contributing to the area of demographic evaluation. Studies on metabolism and mechanisms of action in susceptible and nonsusceptible species, and in man, are essential for extrapolation of animal data to man and for estimation of risk.

Proposed Course: Experiments will continue along the lines suggested by the objectives outlined above. Studies on the comparable metabolism of aflatoxins may help to delineate the mechanisms involved in rather wide species differences in response to these compounds. Similarly, studies on the biochemical effects, toxicity and carcinogenicity of analogs and metabolites may provide evidence on active forms of the agent and on sites of action. Identification of new mycotoxins from other molds isolated from food samples will provide further definition of the scope and character of the potential hazards to man represented by these toxins.

Date Contract Initiated: June 25, 1962

Current Annual Level: $208,025

STANFORD RESEARCH INSTITUTE (NO1-CP-33394)

Title: In Vitro DNA Repair as a Potential Prescreen for Chemical Carcinogens

Contractor's Project Directors: Dr. W. Andrew Maxwell
Dr. M. Barnett

Project Officer (NCI): Dr. Lionel Poirier

Objectives: The objective of this project is to determine the efficacy of utilizing microbial mutation and/or unscheduled DNA synthesis (repair synthesis) in mammalian cells as methods of identifying chemicals which are carcinogenic. If either or both of these systems proves to be accurate in identifying carcinogens and precarcinogens by their mutagenic or DNA damaging activity and is also able to distinguish between carcinogens and analogous noncarcinogens, then they will become part of a battery of mutagenicity assays used as prescreens of environmental chemicals for their potential carcinogenicity.

Major Findings: (1) Microbial Mutation - Most of the direct acting carcinogens appear to affect either Salmonella typhimurium TA1535 or TA1538 when tested in vitro. Mitotic recombination in Saccharomyces cerevisiae D3 is also increased by the direct acting carcinogens. Precarcinogens have been activated to mutagens by adding a postmitochrondrial activating system to the in vitro assay. Noncarcinogens have generally given negative results in both in vitro assays. Preliminary results have also been obtained from the host-mediated assay. (2) Unscheduled DNA Synthesis - This assay system is being tested with a selected group of 85 carcinogens, related noncarcinogens, and mutagens. It has been found that both human lymphocytes from peripheral blood and human diploid fibroblasts, WI-38 cells, undergo unscheduled DNA synthesis following challenge by most direct carcinogenic compounds studied, as well as by precarcinogens which have been activated by a microsomal system. However, the majority of the noncarcinogens studied do not induce repair synthesis, either in the presence or absence of the metabolic activation system. Preliminary results from this laboratory confirm these observations.

Significance to Biomedical Research and the Program of the Institute: Effective prevention is one of the goals of the National Cancer Institute in its effort to control cancer in humans. Modern technology has created a vast pool of chemicals which invade man's environment daily. As rapid testing procedures, the microbial mutation and unscheduled DNA synthesis assays should be of vital importance in screening as many of these substances as is feasible. Chemicals showing mutagenic activity in these assays would be extensively tested in animal assays for carcinogenicity. Appropriate action would then be taken to control human exposure to those chemicals determined to be carcinogenic.

Proposed Course: Studies on the correlation between the carcinogenic and mutagenic activities of the 85 compounds should be concluded during the coming year; whenever feasible, modification of the standard protocols will be undertaken to increase the degree of correlation.

Date Contract Initiated: June 13, 1973

Current Annual Level: $115,421

TEMPLE UNIVERSITY (NO1-CP-51029)

Title: A Search for Carcinogens among Mycotoxins

Contractor's Project Director: Dr. Fritz Blank

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: This contract is aimed at identifying possible carcinogens from fungi pathogenic to man. The original screening program included various extracts and fractions from 17 fungal species. Presently, efforts are being concentrated on the carcinogenic activity of Candida parapsilosis and Candida albicans. These species are the most often isolated pathogenic fungi from clinical specimens as well as having the highest carcinogenicity in the preliminary screening program.

Major Findings: Mycelia of fungi are produced and lyophilized by Dr. F. Blank at Temple University. Extraction of the mycelia (hexane, chloroform, and hot and cold methanol) and fractionation of these extracts for bioassay is performed by Dr. G. Just at McGill University. Bioassays are done by Dr. S.J. Mann at Temple University. In CFW female mice, injections of high doses of hot and cold methanol extracts of Candida parapsilosis gave an increase in the frequency of sarcomas at the site of injection. In Strain A female mice, injections of the hot methanol extract gave a significant increase in the frequency of lung tumors. A scheme for the separation and chemical identification of the major fractions of Candida parapsilosis has been developed.

Significance to Biomedical Research and the Program of the Institute: This study deals with potential carcinogenic fungal metabolites which may be of importance to man through contact or presence in food or other commodities. Such metabolites may be responsible for some of the "spontaneous" cancer incidence in the population.

Proposed Course: The largest number of sarcomas was induced by certain extracts of Candida parapsilosis. When repeated, fewer sarcomas were obtained as a result of changed growth conditions. The original method of growing the fungus was in small Erlenmeyer flasks, whereas the later production was done in a fermentor to increase fungal yields. Candida parapsilosis was, therefore, grown in Erlenmeyer flasks on shakers according to the original conditions. The shaker-grown mycelia, as well as fermentor-grown mycelia, was extracted and fractionated. The various extracts are being bioassayed in CFW female mice, and the experiments will be terminated in June, 1974.

Date Contract Initiated: June 12, 1965

Current Annual Level: $45,188

SUMMARY REPORT

CHEMISTRY & MOLECULAR CARCINOGENESIS SEGMENT

July 1, 1973 through June 30, 1974

The major objectives of the Chemistry & Molecular Carcinogenesis Segment center on gaining an understanding of the molecular process involved in chemical carcinogenesis and an understanding in chemical and biological terms of the important events that lead to carcinogenesis. These studies have been grouped into the following five major approach areas: 1) biochemical properties of chromatin and nucleic acids; 2) study of surface membranes and the manner in which they participate in the regulation of cellular properties; 3) study of polycyclic hydrocarbons and the significance to carcinogenesis of the many products which result from their enzymatic activation; 4) study of the genetics of somatic cells, the chemically induced mutations that can be induced in them, and the significance of this process in carcinogenesis; and 5) studies on haptoglobin types in cancer of the pancreas.

Biochemical Properties of Chromatin and Nucleic Acids

Important studies on DNA repair have been conducted by Dr. Setlow at Oak Ridge National Laboratory (AEC-ORNL) (#Y01-CP-40013) which emphasize the importance of repair process in carcinogenesis. They are presently investigating UV-induced tumors, and report that the formation of thymidine dimers can lead to neoplastic transformation. In studies on non-histone protein fractions new methods have been developed to separate the bulk of these proteins from nucleo-histones in rat liver by Dr. Roth, University of Connecticut (#NIH-NCI-E-72-3268). The method avoids the use of high salt concentrations and urea. Dr. Hnilica at the University of Texas (#N01-CP-43270) has demonstrated tissue specific fractions of the non-histone protein component from rat liver and Novikoff hepatoma. Neoplastic transformation changes antigenic specificity of these proteins to a new type, characteristic of cancer tissues. These studies as a group help in the understanding of the role of altered genetic function in carcinogenesis.

Several new hydro gel systems have been developed by Dr. Halpern of Polysciences, Inc. (#NIH-NCI-E-72-3245). These gels are of large pore and suitable for the study of large molecules. He reports promising results in the separation of high molecular weight DNA by gels containing fixed cationic groups.

Surface Membranes

Professor Friesen at McGill University (#NIH-NCI-E-72-3295) has continued his studies on the preparation of prolactin, the hormone which acts on the surface membrane of mammary tissues. The principal goal of the contract is to provide human prolactin isolated from amniotic fluid for use in studies in human carcinogenesis. In addition, he has characterized prolactin receptors in rat mammary tumors and shown a good correlation between the presence and amount of prolactin receptors and the biological response or dependence of the tumor

on prolactin. A knowledge of the properties of the membrane in normal and malignant cells may lead to an understanding of the mechanism by which malignancies develop, as well as suggesting means of controlling the growth of malignant cells.

A project on carcinogenesis and control of cell cycle is supported directly from the Office of the Associate Director.

Organic Synthesis of Reference Compounds for Study of Metabolic Products of Polycyclic Hydrocarbons

During the past year Midwest Research Institute (#NO1-CP-33387) has labored to overcome some very difficult synthetic problems. The past five months have shown the results of their industry and cooperation with NCI. Thirteen benzo(a)pyrene derivatives and five methylcholanthrene derivatives are completed or near completion, and the three other hydrocarbons in the program are about to begin synthesis. At the same time, all pertinent physical and chemical characteristics have been determined for these compounds for use of investigators to whom the compounds are sent. To date, twenty laboratories have received polycyclic hydrocarbon derivatives.

Omni Research (#NO1-CP-33386) has been developing optimal methods for tritiating the synthesized polycyclic hydrocarbons. At present they are modifying industrial procedures so hydroxylated hydrocarbons will not be destroyed during the exchange process.

The University of Chicago (Dr. R. Harvey) (#NO1-CP-33385) has completed synthesis of eight compounds including five in the DMBA and BA series. Dr. Harvey's work has included stabilizing labile diols by derivatizing to diacetates. He is currently studying diol synthesis procedures offering better yields than the osmate ester technique.

At the University of Texas, Dr. T. Gibson (#NO1-CP-33384) has been studying mutant strains of pseudomonas putida which will metabolize polycyclic hydrocarbons. A strain of beijerinckia has been isolated which produces dihydrodiols from benzo(a)pyrene and benz(a)anthracene.

Enzymatic and Metabolic Studies of Polycyclic Hydrocarbons

Dr. Calvin at the University of California (AEC-University of California) (#YO1-CP-30211) has detected nonenzymatic reactions between polycyclic hydrocarbons and certain nucleotides as a basis for further work on studying carcinogenesis by this class of compounds. In addition, he has developed various lines of epithelial cells which show pronounced differences in the ability to react with polycyclic hydrocarbons. This biological material can be expected to be helpful in determining the significance of aryl hydrocarbon hydroxylase levels in the development of malignancy. Drs. Levin and Adams at Johns Hopkins University (#NO1-CP-33276) are studying the cytochrome P-450 component of the hepatic microsomal system involved in the activation and detoxification of carcinogens. They have identified a component of liver cell sap which transfers heme from the mitochondria, where it is synthesized,

to the microsomes, where it is incorporated into the enzyme. They have developed an assay for this component. Dr. Brookes at the Chester Beatty Institute for Cancer Research (London, England) (#NO1-CP-33367) has compared the DNA products formed from the in vivo interaction of hydrocarbons and DNA with those obtained by reaction with chemically prepared K-region epoxides. The latter compound had been thought to be the biologically generated active compound. There were no common products, casting doubt on the hypothesized role of epoxide.

Dr. Sachs at the Weizmann Institute of Science (Rehovot, Israel) (#NCI-E-70-2217) has been studying the mutagenicity of polycyclic hydrocarbons using mammalian cells. It is desirable to use the Chinese hamster cells, in which susceptibility and resistance to azaguanine is a much studied mutation. Because these cells do not metabolize polycyclic hydrocarbons, they are co-cultivated with rodent cells which are metabolically active. Under these conditions mutations have been observed. Dr. Revel and coworkers at the Weizmann Institute (#NO1-CP-33220) are investigating whether alterations in the translation mechanism of mRNA play a role in carcinogenesis. The contractor has isolated mRNA for the SV_{40} major capsid protein (VP-1) using chromatography over oligo(dT)-cellulose and selective hybridization to SV_{40} DNA. Translation of this mRNA in an in vitro protein synthesis system and characterization of the protein product demonstrated VP-1. A species of His-tRNA has been shown to be encoded in the Mengo Virus genome.

Somatic Cell Genetics

Dr. Siminovitch at the Ontario Cancer Institute (#NIH-NCI-72-2051) has obtained a number of additional mutants of Chinese hamster ovary cells. Although many of them have been characterized to some extent, one mutant in particular has been studied intensively and is most probably due to a temperature-sensitive leucine synthetase. Dr. di Mayorca of the University of Illinois (#NIH-NCI-E-72-3303) has extended his previous findings of the temperature dependence of the transformed genotype of BHK cells, which have been transformed by benzo(a)pyrene and by nitroquinolines. He interprets his results to indicate that the mutations are point mutations and not frame-shift mutations. Dr. Basilico at New York University (#NO1-CP-33279) has isolated more than sixty temperature-sensitive mutants of a Syrian hamster line, BHK, and characterization has begun. A mutant isolated last year and studied further this year has been found to have an impairment in the processing of the 32S ribosomal RNA precursor. In addition to these contracts, a new contract for the production of haploid cells and a new contract for the study of specific mutations in the purine pathway have been initiated.

Haptoglobin Types and Cancer of the Pancreas

Dr. Altschuler at the Home for the Jewish Aged (#NO1-CP-33274) and Dr. Anderson at the M. D. Anderson Hospital (#NIH-NCI-E-71-2268) are completing studies which show a substantial increase in the frequency of the haptoglobin 1 gene in patients with cancer of the pancreas as compared with controls. Dr. Anderson reports that the average age at diagnosis is correlated to some extent with haptoglobin type in that haptoglobin 1-1 type

individuals come to diagnosis approximately 10 years earlier than haptoglobin 2-2 individuals. The combined results suggest that haptoglobin 1-1 allele is increased in pancreatic cancer and in some way enhances the development of adenocarcinoma of the pancreas.

CONTRACT NARRATIVE

CHEMISTRY AND MOLECULAR CARCINOGENESIS SEGMENT

July 1, 1973 through June 30, 1974

AEC-NCI INTERAGENCY AGREEMENT (University of California at Berkeley), (Y01-CP-30211)

Title: Molecular Processes Involved in the Carcinogenic Action of Polycyclic Aromatic Hydrocarbons

Contractor's Project Director: Dr. Melvin Calvin

Project Officers (NCI): Dr. Harry V. Gelboin
Dr. Andrew C. Peacock

Objectives: To define the chemically reactive positions of polycyclic hydrocarbons and to determine the interaction of the reactive intermediates with cell macromolecules and the nature of interaction with oncogenic viruses.

Major Findings: Investigations were begun of the complexing of polycyclic aromatic hydrocarbons (PAHs) with synthetic and natural polynucleotides. A series of experiments studying the interaction of pyrene, benzo(a)pyrene (BP) and benzo(e)pyrene with different nucleotide systems were completed. The method of analysis has been primarily spectroscopic. The question that is being asked is whether an alteration in the excitation and/or emission spectra of these PAHs can be detected when they are examined in the presence of various polynucleotide systems. These shifts in spectra would indicate that a physical interaction between the PAH and the polynucleotide system was occurring. The only significant amount of interaction that can be seen using this technique is between BP or benzo(e)pyrene and poly G. No interaction was seen between pyrene and any of the polynucleotide systems. The next step to be pursued is to look at the binding of BP to polynucleotide systems by following radioactively labeled BP.

Techniques have been developed to study the interaction of PAHs with biological macromolecules. Any investigation of how PAHs interact with biological systems is difficult because of the complexity of the interactions and the number of components involved. Attempts to overcome some of these difficulties will be made by studing the interaction of PAHs with viruses. SV40 and polyoma are well-characterized viruses composed mainly of DNA and protein. Both the DNA and the protein components have been isolated and studied. The experiments to be undertaken necessitate growth of the virus in cells that can metabolize the PAHs. Unfortunately, the monkey cells which support the growth of SV40 do not metabolize the BP; however, mouse epithelial cells actively metabolize the carcinogen. They do not support the growth of SV40, but polyoma virus will multiply in these cells. Purification of polyoma virus grown on these epithelial cells is underway. This system will be used to try to answer the following questions: Do PAHs effect

the production of either RNA or DNA type viruses? What are the effects of PAHs on the number of DNA breaks as measured by the ratio of supercoiled DNA to relaxed circular and linear DNA? Can a chemical linkage be demonstrated between carcinogen and DNA and/or proteins of polyoma? And, finally, what is the effect of inhibitors of PAHs' metabolism on the interaction of PAHs with viruses?

The action of BP on different cell lines of both epithelial and fibroblastic origin has been studied. There have been a number of reports that the cytotoxicity of BP and other PAHs to different cell lines is a function of the ability of the cells to metabolize the hydrocarbon to hydroxylated derivatives. The relationship between cytotoxicity of hydrocarbons and their ability to transform cells is not clear. It is clear, however, that the aryl hydrocarbon hydroxylase (AHH) is involved with transformation. Experiments indicate that different cell lines respond differently to BP in their growth medium and that all the epithelial cell lines tested were more sensitive to the carcinogen than the fibroblastic cell lines.

The metabolism of PAHs in various cell lines has also been studied. In general, it was found that cells showing cytotoxicity to hydrocarbons and induction of the AHH also are capable of converting the hydrocarbons into fluorescent derivatives which can be detected in the cells and in the medium surrounding the cells. Following these observations, attempts were made to develop a method of assaying hydrocarbon metabolism in single cells by measuring the amount of fluorescence of the metabolic products in each cell. The technique is a modification of flow microfluorimetry and uses a laser tuned to the UV to excite the derivatives which, at alkaline pHs, fluoresce at wavelengths well separated from that of the parent compound. To date, this green fluorescence, both by fluorescent microscopy and flow microfluorimetry, has been detected. Some difficulties have been experienced with cell handling at alkaline pHs in that there is considerable clumping and cell lysis. Attempts will be made to develop optimal conditions for the fluorescence to appear and the cells to remain intact.

Significance to Biomedical Research and the Program of the Institute: The widespread distribution of polycyclic hydrocarbons as a result of the combustion of fossil fuel, both in moving and in stationary power plants, as well as in the combustion of agricultural wastes, makes them important environmental carcinogens. Their presence as a principal component of tobacco smoke, whose carcinogenicity has been clearly demonstrated epidemiologically, makes it even more critical to know their mode of action--the role of enzymes in the activation as well as in the binding of the carcinogen, the cellular components and the components of oncogenic viruses. A first step has already been taken in helping to understand how these hydrocarbons may become active. Furthermore, the possible detection of their first stage of activity by induction of enzymes such as aryl hydrocarbon hydroxylase or reverse transcriptase would be a realistic diagnostic tool for controlling cancer. An indication that there may be methods for effective prevention using a specific enzyme inhibitor has appeared in the demonstration that certain rifamycin derivatives given by suitable means can indeed slow down the appearance of hydrocarbon induced mammary tumors. The possibility of an effective treatment is always before us, particularly in terms of the cell membrane changes.

Proposed Course: A number of projects all designed to approach the question of the metabolic activation of polycyclic aromatic hydrocarbons and the subsequent transformation of mammalian cells has been proposed. This research is interdisciplinary in nature and, consequently, approaches a similar problem from a number of different directions.

Date Contract Initiated: April 12, 1971

Current Annual Level: $166, 000

AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory) (N01-CP-40013)

Title: NCI-AEC Carcinogenesis Program

Contractor's Project Director: Dr. Francis T. Kenney

Project Officer (NCI): Dr. Allen Heim

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis.

CHICAGO, UNIVERSITY OF (NO1-CP-033385)

Title: Organic Synthesis of Cold and Tritiated Polycyclic Hydrocarbon Derivatives

Contractor's Project Director: Dr. Ronald G. Harvey

Project Officer (NCI): Dr. Harry Gelboin

Objectives: The overall objective is to synthesize polycyclic hydrocarbons to be available to the scientific community for *in vivo* and *in vitro* studies in carcinogenesis. They can be considered to be of special value in studying enzyme pathways in chemical carcinogenesis.

Major Findings: Progress in attaining the synthetic objectives of this project has exceeded expectations. Synthetic accomplishments include: (1) synthesis of the K-region epoxides, cis and trans diols, and quinones of benz(a)anthracene (BA), benzo(a)pyrene (BP), and 7,12-dimethylbenz(a)anthracene (DMBA) *via* the general method previously developed in this laboratory; (2) extension of this general approach to the synthesis of the K-region phenols (both isomers) *via* the corresponding dioldiacetates, trifluoracetates, mesylates, or monoacetates of BA, BP, and DMBA; (3) development of efficient methods for reconversion of the cis and trans dioldiacetates (more stable and readily purifiable) and phenol acetates to the free diols and phenols (air sensitive), respectively; (4) cleavage of 5-AcO-DMBA with methyllithium afforded as the major product 5-keto-5,6-dihydro-DMBA, indicating a remarkable preference of the keto over the phenol tautomer of 5-HO-DMBA; (5) development of a novel synthesis of K-region quinones from the corresponding dihydro compounds by mild oxidation with dichromate in acetic anhydride-acetic acid (eliminating the need for the

costly and hazardous OsO_4 reagent); (6) synthesis of K-region and non-K-region hydroquinones (previously unknown) isolated as the diacetates through reduction of the corresponding quinones with "aged" borohydride in glyme; (7) synthesis of 7-hydromethyl-12-methyl-BA (and the related 7-methyoxymethyl- and 7-acetoxymethyl derivatives) from 7,12-dimethoxy-7,12-DMBA through a novel acid catalyzed rearrangement reaction encountered during repetition of the Bachmann synthesis of DMBA; (8) exploration of methods to allow the synthesis of specifically tritium-labelled K-region oxidized derivatives of BP; (9) exploration of methods for the direct synthesis of the K-region epoxides from the cisdiols; and (10) miscellaneous syntheses (6-AcO-BP, 6-HO-BP, 1-AcO-6-Br-BP [precursor of 1-AcO-BP], BP-1,6- and 3,6-quinones, "BA-5,6-dialdehyde", etc.).

All the above reactions were initially conducted on a small (5-100mg) scale until a satisfactory procedure affording acceptable yields of pure products could be devised. All compounds were characterized by nmr spectra, supplemented by tlc, mp, IR, UV, mass spectra, as appropriate. Satisfactory microanalysis has been obtained on many, though as yet not all, of the new compounds. The following compounds have been synthesized on larger scale, and quantities 1g or larger are now on hand awaiting packaging: BP-4,5-cisdiol diacetate, BP-4,5-oxide, BA-5,6-cisdiol, BA-5,6-transdiol diacetate, BA-5,6-oxide, DMBA-5,6-cisdiol, DMBA-5,6-oxide, and 7-hydroxymethyl-12-methyl-BA.

Significance to Biomedical Research and the Program of the Institute: This is one of four contracts designed to prepare 1 gram in 1 mg samples of 44 compounds consisting of known or suspected chemical carcinogens for the purpose of making these compounds available to the scientific community throughout the world. It would be virtually impossible for a laboratory lacking specific synthetic experience to synthesize any one of these compounds for a specific experiment. The combined contract will actually put the National Cancer Institute in the position of providing compounds for pertinent experiments in chemical carcinogenesis which could not be carried out otherwise.

Proposed Course: Compounds for which syntheses have been devised on small scale (or cold in the case of tritium-labelled derivatives) will be prepared on the required scale. Exploratory experiments to develop convenient syntheses of other compounds soug ht will continue. Particular emphasis will be given development of regioselective syntheses of K-region phenol isomers and utilization of 270 MHz nmr spectroscopy to assign isomer structure eliminating the necessity for multistep unequivocal syntheses. Improved techniques of separation and purification of these sensitive molecules, particularly pressure liquid chromatography will be investigated.

Date Contract Initiated: June 20, 1973

Current Annual Level: $72,000

CONNECTICUT, UNIVERSITY OF (NO1-CP-23268)

Title: Isolation and Characterization of Non-Histone Proteins Binding DNA

Contractor's Project Director: Dr. Jay S. Roth

Project Officer (NCI): Dr. C. Wesley Dingman

Objectives: The primary objective of this project is to develop standardized reliable techniques for isolating non-histone proteins that show significant affinity for homologous DNA.

Major Findings: Two new methods have been developed which separate the bulk of the non-histone chromosomal protein from the nucleohistone of rat liver under very mild conditions. The methods involve dissolving of rat liver chromatin in 0.6M NaCl or KCl with 10 mM phosphate pH 8.0 to dissociate about 90% of the non-histone proteins away from the undissociated DNA and histone. This is followed by either exclusion chromatography on agarose gel or on hydroxyapatite. Both methods avoid high salt concentration and use of urea and are very rapid and simple.

Significance to Biomedical Research and the Program of the Institute: The non-histone DNA binding proteins are believed to play a major role in the control of gene activity. Methods which allow the rapid preparation of these proteins in their native state will enable them to be purified, characterized, and their function determined. This could be of importance in understanding gene control in normal and cancer cells.

Proposed Course: The isolated proteins will be passed through DNA affinity columns and the bound proteins fractionated and characterized. Significant differences will be sought between DNA-binding proteins isolated from normal and malignant cells.

Date Contract Initiated: June 29, 1972

Current Annual Level: $34,646

HOME FOR THE JEWISH AGED (NO1-CP-33274)

Title: Study of Serum Haptoglobin Types in Patients with Carcinoma of the Pancreas

Contractor's Project Director: Dr. Henry Altschuler

Project Officer (NCI): Dr. Andrew Peacock

Objectives: The objectives are (1) to identify individuals who because of their genetic makeup may be liable to an increased risk of acquiring carcinoma of the pancreas; (2) to determine whether genetic characteristics believed to have significance with respect to leukemia and probably breast and lung cancer are also significant in cancer of the pancreas.

Major Findings: Serum samples have been collected from individuals with the diagnosis of cancer of the pancreas and from other individuals, both healthy and with various disease processes, to constitute control groups. The serum haptoglobin types have been determined for each serum and a determination made whether the group with pancreatic carcinoma differs from the control groups with respect to the distribution of haptoglobin type and the frequency of the haptoglobin 1 gene. In addition, the ABO blood group distribution of the individuals belonging to these various groups has been determined. The contractor has typed for haptoglobin, up to the present time, 86 patients with diagnosis of carcinoma of the pancreas, 60 patients with conditions simulating carcinoma of the pancreas, and about 1,000 controls a high cancer risk population. The results indicate that a predominance of Type 1-1 haptoglobin is found among the patients with cancer of the pancreas, as compared to the control groups. This becomes more evident when the patients are divided according to sex.

Significance to Biomedical Research and the Program of the Institute: There is an increasing need to define differences between individuals who are susceptible to some form of malignant disease and those who are not. The project may, therefore, be helpful in establishing a possible marker characteristic of an increasingly frequent form of cancer for which very few etiological clues currently exist and which often manifests itself clinically only when already far advanced. The project is also attempting to determine whether these genetic characteristics, currently believed to be significant with relation to leukemia, are also significant in cancer of the pancreas.

Proposed Course: To continue collecting serum samples from patients with carcinoma of the pancreas and from control groups. By the end of the contract period, the collected data will be tabulated for computer analyses on both the haptoglobin type and the blood ABO group distribution and analyzed statistically to determine if there are significant differences among the groups studied.

Date Contract Initiated: April 29, 1971

Current Annual Level: $ 0

ILLINOIS, UNIVERSITY OF (NO1-CP-23303)

Title: Temperature Sensitive Mutants in *In Vitro* Carcinogenesis

Contractor's Project Director: Dr. Giampiero di Mayorca

Project Officer (NCI): Dr. John Bader

Objectives: (1) To define a system for the study of chemical carcinogenesis in cell culture, (2) to identify cellular response to a variety of known carcinogens, (3) to quantify the transformation observed, (4) to analyze the biochemical defects responsible for transfomation, and (5) to demonstrate by back mutation experiments that malignant transformation is induced by a single point mutation.

Major Findings: The temperature dependence of the transformed genotype of BHK_{21} cells has been extended to cells transformed by benzo(a)pyrene and by nitroquinolines. A temperature-dependent transformed genotype has been observed in some of the clones of FIII (rat fibroblast) transformed by nitroquinoline; however, about 50% of both the BHK and FIII clones were found to be transformed without any temperature restriction. This finding is important because nitroquinolines are known to cause in bacteria both point mutations and frame shift mutations. Alkylating agents as nitrosamines are known to cause only point mutations. The fact that one obtains a great preponderance of the temperature-dependent transformed genotype with nitrosamines and both temperature-dependent and temperature-independent transformed genotype with nitroquinoline in roughly the same proportion supports the working hypothesis of a mutational mechanism for transformation. Frame shift mutations result in nonsense, and never result in a conditionally active molecule.

Significance to Biomedical Research and the Program of the Institute: An exploitable system for the determination and analysis of chemical carcinogens is essential to progress in understanding carcinogenesis with the ultimate view to preventing and/or curing tumors induced by chemicals.

Proposed Course: The quantitation of chemical carcinogenesis in cell cultures will be developed. The experimentation will be extended to simian and human cells. Back mutation experiments will be performed and pure frame shift mutagens will be employed with the purpose of providing further evidence in favor of mutational mechanisms in chemical carcinogenesis.

Date Contract Initiated: June 20, 1972

Current Annual Level: $161,695

INSTITUTE OF CANCER RESEARCH (NO1-CP-33367)

Title: Nature of the Polycyclic Hydrocarbon--Nucleic Acid Compound in Hydrocarbon Carcinogenesis

Contract Project Director: Dr. Peter Brookes

Project Officer (NCI): Dr. Harry V. Gelboin

Objectives: To understand the chemistry of the reaction between carcinogenic hydrocarbons and the DNA of mammalian cells which results when these compounds are metabolized by the cells. Furthermore, since it now seems probable that more than one reaction product is present in the DNA, the significance of each reaction will be considered in relation to mutagenesis and transformation.

Major Findings: DNA isolated from mouse embryo cells which had been exposed to tritium labelled benzo(a)pyrene (BP) has been enzymically degraded to deoxynucleosides and the products separated by column chromatography. These products were compared with those obtained by reaction of the K-region epoxide of BP (supplied by University of Chicago [33385]) with DNA. The lack of any

common product was in agreement with similar earlier findings for 7-methylbenz(a)anthracene and its K-region epoxide.

The indication from earlier work that the major DNA bound product involved the pyrimidine bases of the nucleic acid has been reinvestigated by pre-labelling the DNA of mouse embryo cells with the appropriate purine or pyrimidine precursors before treating the cells with 7-methylbenz(a)anthracene. Results so far seem to contradict the original idea of pyrimidine binding.

While it has been established that a liver microsomal preparation can mediate the interaction of BP with DNA, the relation of this binding to that observed in cells is unknown. Preliminary experiments have shown DNA to be a powerful inhibitor of BP hydroxylation by microsomes. The observed hydrocarbon-DNA binding may, therefore, not be typical of the non-inhibited enzyme system.

The possible role of a hydrocarbon radical or radical cation in cellular nucleic acid binding has been investigated by activating 7MBA or BP by a number of one-electron oxidants in the presence of DNA. Degradation and fractionation of the resulting DNA in the usual way has so far failed to yield any product identical to those obtained from DNA with in vivo bound carcinogen.

A program for the synthesis of a number of BP - nucleoside derivatives has been initiated to provide appropriate reference compounds for the DNA-BP studies mentioned above.

It has been shown that the BHK21/C13 cell line will metabolize carcinogenic hydrocarbons with consequent DNA binding. The induction of azaguanine resistant mutants in this cell line will enable the mutagenic potential of the hydrocarbon-DNA reaction to be assessed.

Significance to Biomedical Research and the Program of the Institute: A complete understanding of the chemistry of the in vivo reaction between carcinogenic hydrocarbons and DNA will indicate the nature of the reactive species mediating this reaction and, hence, suggest ways in which the production of this moiety could be inhibited. The nature of the genetic alterations consequent on hydrocarbon-DNA reaction, as revealed by mutation studies, should advance understanding of the molecular basis of neoplasia and further advance knowledge of the types of compound which might be expected to illicit this response.

Proposed Course: To proceed with the various aspects of the program as outlined above using the analytical methods and cell culture systems which have recently been developed.

Date Contract Initiated: June 25, 1973

Current Annual Level: $65,000

THE JOHNS HOPKINS UNIVERSITY (NO1-CP-33276)

Title: Regulation of the Heme Moiety of P-450 in Relationship to Carcinogen Metabolizing Activity

Contractor's Project Directors: Dr. Ephraim Y. Levin
Dr. Peter R. Adams

Project Officer (NCI): Dr. Harry V. Gelboin

Objectives: To determine the mechanism of regulation of the synthesis and degradation of the heme moiety of P-450, and to examine this regulation with respect to carcinogen metabolism.

Major Findings: In vitro studies of microsomal heme synthesis from labeled precursors have shown the presence of a component in liver cell sap which transfers mitochondrially-synthesized heme to microsomes. A method of assay for this component, which seems to be protein in nature, has been developed. Over 80% of the activity for the transfer of heme was retained on dialysis, but over 90% was lost by boiling or by incubation with trypsin. Over 99% of the activity was excluded from Sephadex G-25. Fractionation with ammonium sulfate and chromatography on Sephadex G-100 failed to purify the activity further; all fractions had approximately the same specific activity. However, despite this evidence for the non-specificity of the transfer process, it is estimated that less than 5% of the total protein molecules in the cell sap can bind to heme from mitochondria. Moreover, pure samples of bovine serum albumin, human α-globulin and bovine prothrombin are inactive in concentrations at which cell sap is active.

Significance to Biomedical Research and the Program of the Institute: Cytochrome P-450 is a component of the hepatic microsomal system involved in the activation and detoxification of carcinogens. Induction of this system has been shown to be linked with simultaneous induction of increased synthesis of its heme prosthetic group. The current studies have focused on the transfer of newly formed heme from its mitochondrial site of synthesis to its apoprotein in microsomes. This transfer step is a possible site for regulation of the relative amounts of heme in the two subcellular compartments.

Proposed Course: Attempts will be made with other methods of protein fractionation to purify and characterize the heme transfer activity of cell sap. In addition, tests will be made with various purified proteins for heme transfer activity in order to determine what specific properties are associated with activity. It is hoped to determine what controls the response of microsomal heme synthesis to the presence of xenobiotic compounds, and whether transfer activity is involved in this control. A study of the relationship between the induction of microsomal heme synthesis and the induction of microsomal cytochrome P-450 will be initiated.

Date Contract Initiated: April 30, 1971

Current Annual Level: $83,980

MANITOBA, UNIVERSITY OF (Formerly: McGill University) (NO1-CP-43250)

Title: Isolation, Purification and Characterization of Human Prolactin

Contractor's Project Director: Dr. Henry Friesen

Project Officers (NCI): Dr. Andrew C. Peacock
Dr. Marie Green

Objectives: To compare amniotic fluid and pituitary gland as a source of human prolactin and to provide purified human prolactin for distribution to investigators studying mammary cancer.

Major Findings: Amniotic fluid may be an excellent source of prolactin. Preliminary studies have shown that affinity chromatography will be a convenient method for purification. Analyses of amino acid composition and amino acid sequence of a portion of amniotic fluid prolactin indicate that its sequence is identical to that of pituitary prolactin. Binding sites for prolactin have been investigated in tissues. Preliminary studies indicate that there is a good correlation between the presence and amount of prolactin receptors and the biological response or dependence of rat mammary tumors on prolactin.

Significance to Biomedical Research and the Program of the Institute: Prolactin is a major factor in determining growth and differentiation of mammary tissue. In some experimental animals prolactin levels strongly influence the development of the so-called "hormone dependent" tumors. The role of prolactin in human disease is less clear. These studies will assist in clarifying the problem.

Proposed Course: Continued experimentation and evaluation of new procedures for the production of human prolactin and further investigation of its role in breast cancer.

Date Contract Initiated: June 30, 1972

Current Annual Level: $57,145

MICROBIOLOGICAL LABORATORIES, INC. (NO1-CP-33388)

Title: Organic Synthesis of Cold and Tritiated Polycyclic Hydrocarbon Derivatives

Contractor's Project Director: Dr. Lester Friedman

Project Officer (NCI): Dr. James Selkirk

Objectives: To synthesize polycyclic hydrocarbon derivatives to study metabolic activation of carcinogens and to supply outside research laboratories with these compounds for their investigation.

Major Findings: No major findings to report at this time.

Significance to Biomedical Research and the Program of the Institute: The compounds being synthesized are significant inasmuch as they are areneoxides or other possible primary or secondary intermediates in the oxidative metabolism of aromatic substrates. Many of these substances have generated much interest since they have been implicated as the causative agents in the carcinogenic, toxic, and/or mutagenic activity displayed by many aromatic hydrocarbons.

Proposed Course: The proposed course of synthesis is designed to afford relatively large quantities of postulated oxidative metabolic intermediates (from their corresponding aromatic hydrocarbons) in high yields with a minimum of contamination.

Date Contract Initiated: June 30, 1973

Current Annual Level: $25,575

MIDWEST RESEARCH INSTITUTE (NO1-CP-33387)

Title: Organic Synthesis of Cold and Tritiated Polycyclic Hydrocarbon Derivatives

Contractor's Project Director: Mr. James F. Engel

Project Officer (NCI): Dr. James Selkirk

Objective: The overall objective is the synthesis of derivatives of polycyclic hydrocarbons to be available to the scientific community for *in vivo* and *in vitro* studies in carcinogenesis. These compounds are considered to be of special value in the study of enzyme pathways in chemical carcinogenesis.

Major Findings: A total of 46 derivatives of the following five parent polycyclic hydrocarbons have been selected for studies: benzo(a)pyrene, 3-methylcholanthrene, dibenz(a,h)anthracene, benz(a) anthracene, and 7,12-dimethylbenz(a)anthracene. Thus far the syntheses of phenols, quinones, epoxides, dihydrodiols, dialdehydes resulting from the cleavage of vicinal-substituted oxygenated derivatives, and alkyl-substituted parent hydrocarbons have been initiated and a substantial number have been completed. The syntheses of the tritiated analogs of a number of the above compounds are being examined.

Significance to Biomedical Research and the Program of the Institute: This contract is designed to prepare 1 gram quantities of known or suspected metabolites of chemical carcinogens for the purpose of making these compounds

available to the scientific community throughout the world. It would be virtually impossible for a laboratory lacking specific synthesis experience to prepare any one of these compounds for a specific experiment. This contract actually places the National Cancer Institute in the position of providing compounds for pertinent experiments in chemical carcinogenesis which could not be carried out otherwise.

Proposed Course: To continue with large-scale syntheses of cold and tritiated polycyclic aromatic hydrocarbon derivatives and make them available to the scientific community through the Carcinogenesis Program.

Date Contract Initiated: June 15, 1973

Current Annual Level: $192,000

NEW YORK UNIVERSITY MEDICAL CENTER (NO1-CP-33279)

Title: The Isolation, Propagation, and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions

Contractor's Project Director: Dr. Claudio Basilico

Project Officer (NCI): Dr. John P. Bader

Objectives: The objectives of this contract are (1) the isolation and characterization of temperature sensitive (ts) mutants of somatic animal cells and (2) the use of these mutants to study mutation mechanisms in somatic animal cells, and the effect of specific biochemical lesions on the regulation of cell growth and division.

Major Findings: A number (>60) of ts mutants of the Syrian hamster cell line BHK 21/13, including many derived from an HGPRT$^-$ BHK variant, have been isolated and characterization begun. These mutants exhibit low reversion and low leakiness, and, as such, are very suited for biochemical and genetic studies. Treatment with nitrosoguanidine increases the frequency of reversion at least tenfold, suggesting that these mutations are in single genes, possibly originating from simple base substitutions. The ts mutations studied are recessive, as shown by experiments of somatic cell hybridization. Complementation tests have assigned the mutants tested so far to 12 complementation groups, with a few groups containing more than one mutant. Five mutants have been tested for x-linkage and found negative.

An extensive characterization of two of these mutants has been performed. Mutant ts 422E cannot grow at 39° because of a defect in the production of 28S ribosomal RNA, the impairment taking place at the level of processing of the 32S rRNA precursor. The ts gene product involved is probably a ribosomal protein or enzyme. This mutation is truly recessive, as the defect disappears in interspecific hybrid cells which produce hamster 28S RNA at any temperature. Mutant ts AF8 does not synthesize DNA at 39° because of a block in the G1 phase of the cell cycle. Cells at 39° have a 2N DNA content, high cAMP levels

and remain viable for long times. Mitochondrial DNA synthesis is not reduced. Transformation by polyoma virus does not reverse the mutation.

Significance to Biomedical Research and the Program of the Institute: Cancer cells have altered regulation of proliferation and differentiation. Conditional letal ts mutations have proven to be extremely useful for a variety of studies with microorganisms, and their usefulness to further the understanding of somatic animal cells and their regulatory mechanisms is beginning to be demonstrated. Such mutants, in particular those affected in macromolecular synthesis, cell cycle regulation, and possibly membrane formation, should be extremely valuable in studies of carcinogenesis. They should provide an understanding of the cell regulatory mechanisms and of their breakdown during neoplastic transformation, which is basic to our understanding of cancer. In particular, the study of the mutants already characterized is going to provide information of the relationship between ribosomal RNA synthesis and cell division and of the regulatory mechanisms which allow normal, but not transformed cells, to stop in G1 (or GO).

The well-defined characteristics of these mutations should also prove them a valuable tool for investigating the possible mutagenic effects of chemical carcinogens in mammalian cells.

Proposed Course: The proposed course aims at (1) the isolation of additional ts mutants, particularly with the improvement of the selection and mutagenization procedures for the purpose of obtaining specific classes of mutants (DNA synthesis, membranes, etc.); (2) the further characterization of the mutants already obtained, both from the biochemical and genetic point of view; (3) the investigation of the mechanism of the expression of recessive mutations in diploid cells; (4) the use of the mutants for specific experiments aimed at the investigation of the relationship between ribosomal RNA synthesis and cell division, or the study of the host cell functions required for adenovirus multiplication, or polyoma neoplastic transformation; and (5) the investigation of the effects of chemical carcinogens on the reversion rate of ts mutations and/or their expression.

Date Contract Initiated: June 26, 1971

Current Annual Level: $128,949

OHIO STATE UNIVERSITY (NO1-CP-43248)

Title: Research on Preparation of Polymers Containing Carcinogenic Moieties

Contractor's Project Director: Dr. Melvin S. Newman

Project Officer (NCI): Dr. James K. Selkirk

Objectives: To attach hydrocarbons known to be carcinogenic to non-biodegradable polymers by linkages stable to enzymes to find out if such substances will produce cancer in test systems.

Major Findings: As this is a new contract, no major findings can as yet be reported. The synthesis of certain compounds is underway.

Significance to Biomedical Research and the Program of the Institute: If the polymers which are proposed prove to be carcinogenic, it will show that cancer processes can be initiated from without the cell because these polymers will be too large to go through the cell wall.

Proposed Course: The synthesis of 2-diazomethyl-7,12-dimethylbenz(a) anthracene and 10-diazomethyl-7,12-dimethylbenz(a)anthracene are the first targets.

Date Contract Initiated: January 11, 1974

Current Annual Level: $39,988

OMNI RESEARCH INC. (NO1-CP-33386)

Title: Organic Synthesis of Cold and Tritiated Polycyclic Hydrocarbon Derivatives

Contractor's Project Director: Dr. Owen H. Wheeler

Project Officer (NCI): Dr. James K. Selkirk

Objectives: To prepare specifically labelled derivatives of benz(a)pyrene for distribution to scientists studying chemical carcinogens.

Major Findings: A series of tritium-labelled oxygenated derivatives of benz(a)-pyrene are being synthesized from high specific activity tritium-labelled benz(a)pyrene. Benz(a)pyrene-1,6-quinone and benz(a)pyrene-3,6-quinone are obtained as a mixture by oxidation of benz(a)pyrene with chromium trioxide and are separated by chromatography. Hydroxylation of benz(a)pyrene with osmium tetroxide affords cis-4,5-dihydro-4,5-dihydroxybenz(a)pyrene. 6-Hydroxy-benz(a)pyrene is formed from 6-acetoxybenz(a)pyrene, the product of the reaction of benz(a)pyrene with lead tetracetate. Benz(a)pyrene-4,5-dihydro-4,5-epoxide is being synthesized by oxidizing cis-4,5-dihydro-4,5-dihydroxybenz(a)-pyrene to benz(a)pyrene-4,5-quinone, reducing this to trans-4,5-dihydro-4,5-dihydroxy benz(a)pyrene using lithium aluminum hydride, and then ring closing the trans-diol with dimethylformamide dimethylacetal.

Significance to Biomedical Research and the Program of the Institute: This contract will provide necessary labelled derivatives to scientists studying chemical carcinogenesis in laboratories lacking the facilities and expertise for the difficult syntheses of polycyclic hydrocarbons.

Proposed Course: To continue this work to completion as outlined above.

Date Contract Initiated: June 29, 1973

Current Annual Level: $25,000

ONTARIO CANCER INSTITUTE (NO1-CP-22051)

Title: The Isolation, Propagation, and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions

Contractor's Project Director: Dr. Louis Siminovitch

Project Officer (NCI): Dr. John Bader

Objectives: (1) Isolation of cell lines carrying mutations affecting key cell functions such as DNA replication or mitosis, or key cell structures such as the plasma membrane. (2) Biochemical and physiological characterization of the mutant lines. (3) Examination of the properties of hybrid cell lines obtained by fusion of cells of one mutant line with other normal or mutant lines, for complementation analysis and possible mapping. (4) Extension of the techniques, as they are developed, to a variety of cell types chosen not only on the basis of their suitability for studies of the regulation of cell multiplication and differentiation, but also for other purposes, such as the detection of environmental carcinogens and mutagens.

Major Findings: Conditional letal temperature-sensitive mutants of Chinese hamster ovary cells have been selected. All isolates have been characterized in a preliminary way and one isolate has been studied intensively. In this mutant, protein synthesis is defective at the non-permissive temperature, most probably due to a temperature-sensitive leucine synthetase. A method to isolate specific temperature-sensitive mutants for protein synthesis has been developed. One such mutant has been characterized in a preliminary way and involves asparagine metabolism.

Mutants resistant to inhibitors such as ouabain, α-amanitin, colchicine, concanavalin A (Con A) and phytohaemagglutinin (PHA) have been selected. The ouabain mutation is probably co-dominant and its frequency of occurrence has been measured. The mutation to α-amanitin probably involved the structural gene for RNA polymerase, since mutants have normal enzyme levels with altered sensitivity to the drug. Preliminary tests indicate that the mutation is dormant. Colchicine-resistant mutants seem to involve an alteration in the cell membrane. Such mutants show collateral resistance to other unrelated drugs, such as actinomycin D. Con A-resistant mutants also seem to show associated defects, such as temperature sensitivity. Mutants resistant to PHA behave as recessive in somatic cell hybrids, show decreased binding of PHA and increased sensitivity to Con A.

Histidinol-resistant mutants have been found to contain increased levels of the histidinyl-tRNA synthetase.

A variety of auxotrophic mutants have been selected and some of these characterized in a preliminary way.

Significance to Biomedical Research and the Program of the Institute: Cancer may be regarded as a disease in which cells are genetically altered such that their regulation of cellular multiplication and differentiation is defective.

Temperature-sensitive mutations affecting key functions involved in cell growth, and in particular DNA replication and mitosis are of specific interest in respect to obtaining an understanding of the breakdown of regulation during carcinogenesis. Suitably characterized cell lines are useful not only for studies of cellular regulatory processes, but for other purposes as well. One example is the use of cell culture systems for the detection of environmental carcinogens or mutagens. In addition, studies on the genetic basis of drug resistance are of special interest in respect to chemotherapy. For example, our finding that mutation to resistance to colchicine and to Con A engenders collateral changes in sensitivity to other drugs must be considered in contemplation of multiple drug trials.

Proposed Course: To continue with the studies as outlined above.

Date Contract Initiated: January 1, 1972

Current Annual Level: $58,540

POLYSCIENCES, INC. (NO1-CP-23245)

Title: Optimizing Electrophoretic Separation of Proteins with New Hydrogels

Contractor's Project Director: Dr. B. D. Halpern

Project Officer (NCI): Dr. Andrew C. Peacock

Objectives: To develop hydrogel systems specifically suitable for gel electrophoretic separation of biological macromolecules related to carcinogenesis studies. This study is aimed at achieving the gel properties such as (a) large pore size to permit the study of large molecules, (b) desirable associative effects to improve the specificity and resolution, and (c) greater elutability of substances from gels.

Major Findings: Several new hydrogel systems have been developed for the specific objectives. Gels derived from polyethylene glycol 20000 diacrylate and methoxy polyethylene glycol 350/550 monoacrylate have been particularly found to be large pore gels suitable for study of large molecules, as indicated by the electrophoretic mobility of high molecular weight DNA and ribosomal RNA. High molecular weight DNAs ($>10^6$ MW) which are electrophoretically inseparable in ordinary gels, have been found to be resolvable by charge effects in gels containing fixed cationic groups and the separation could be further improved when the cationic gel was used as a small portion at the top. Several gels which are solubilizable by mild chemical or thermal treatment have been developed for application in electrophoresis.

Significance to Biomedical Research and the Program of the Institute: Important elements in the cellular control reside in the macromolecules such as DNA and RNA in the cell. This project will promote the isolation, characterization and study of such molecules.

Proposed Course: The utility of large pore gels for the study of very large molecules such as polyribosomes, viral RNA (70S) and DNA will be evaluated.

The application of "ion-exchange electrophoresis" (e.g., cationic gel electrophoresis) will be explored further, particularly for the fractionation of high molecular weight DNA, transfer RNAs, etc. The application of solubilizable gels for post electrophoretic isolation of macromolecules will be investigated.

Date Contract Initiated: May 17, 1972

Current Annual Level: $58,540

TEXAS, UNIVERSITY OF (at Austin) (NO1-CP-33384)

Title: Growth of Soil Bacteria on Polycyclic Hydrocarbons

Contractor's Project Director: Dr. David T. Gibson

Project Officer (NCI): Dr. James P. Selkirk

Objectives: (1) To explore the possibility of the formation of chemical carcinogens by the action of soil bacteria on polycyclic hydrocarbon precursors; (2) To isolate and identify oxygenated metabolic products formed from polycyclic hydrocarbons by bacteria; (3) To obtain mutant strains of bacteria that accumulate significant amounts of oxygenated products for supply to the scientific community for in vivo and in vitro studies in carcinogenesis; (4) To develop synthetic routes for the formation of arene oxides from microbiologically-produced cis-dihydrodiols.

Major Findings: Several bacterial strains have been isolated that show potential for the degradation of polycyclic hydrocarbons. One of these strains has been identified as a Beijerinckia species. Cells of this organism, after growth in the presence of phenanthrene or biphenyl, oxidize benzo(a)-pyrene and benz(a) anthracene to polar metabolites. A mutant strain (B-836) has been isolated that oxidizes phenanthrene to cis-3,4-dihydroxy-3,4-dihydrophenanthrene. Cells of the mutant strain, after growth in the presence of phenanthrene, oxidize benzo(a)pyrene and benz(a)anthracene to dihydrodiols. The ultraviolet spectrum of the dihydrodiol formed from benzo(a)pyrene is almost identical to that reported for 9,10-dihydroxy-9,10-dihydrobenzo(a)-pyrene. Also, acid-catalyzed dehydration of the bacterial product yields a derivative whose spectral and chromatographic characteristics are identical to those given by 9-hydroxybenzo(a)pyrene. It would appear that Beijerinckia B-836 oxidizes benzo(a)pyrene to cis-9,10-dihydroxy-9,10-dihydrobenzo(a)-pyrene. The same organism also oxidizes benz(a)-anthracene to a product that appears to be cis-1,2-dihydroxy-1,2-dihydrobenz(a)anthracene. The above observations represent the first identification of metabolic products formed from polycyclic hydrocarbons by bacteria.

A mutant strain of Pseudomonas putida has been isolated that oxidizes a variety of monocyclic aromatic hydrocarbons to cis-dihydrodiols. This organism has been used to produced several grams of cis-3,5-dichloro-3,5-cyclohexadien-1,2-diol. In collaboration with Dr. D. M. Jerina of the National Institutes of

Health a method has been developed for the conversion of this compound into an arene oxide. The application of this synthetic route to the synthesis of arene oxides from cis-dihydrodiols of polycyclic hydrocarbons should yield compounds of interest to the National Cancer Institute that would be impossible to prepare by conventional chemical syntheses.

Significance to Biomedical Research and the Program of the Institute: Polycyclic aromatic hydrocarbons have been shown to occur in air, water, and food. Thus they come into contact with all forms of life. At this time there are no detailed reports on the metabolism of these compounds by bacteria. This report documents for the first time the bacterial oxidation of benzo(a)pyrene and benz(a)anthracene to dihydrodiol derivatives. Studies with non-carcinogenic aromatic hydrocarbons would suggest that bacteria use a different enzymatic mechanism to that observed in liver microsomes, for the production of these compounds.

The development of a synthetic route to arene oxides from microbiologically-produced cis-dihydrodiols suggests that it should be possible to prepare several different carcinogens for distribution to the scientific community.

Proposed Course: To proceed with the isolation and identification of the metabolic products formed from polycyclic hydrocarbons by microorganisms. To attempt to produce sufficient quantities of cis-dihydrodiol derivatives for the synthesis of several polycyclic hydrocarbon derivatives which will be made available to the scientific community through the Carcinogenesis Program.

Date Contract Initiated: June 25, 1973

Current Annual Level: $53,500

TEXAS, UNIVERSITY OF (M.D. Anderson Hospital and Tumor Institute)
(MOT-CP-T2268)

Title: Study of Serum Haptaglobin Types in Patients with Carcinoma of the Pancreas

Contractor's Project Director: Dr. David E. Anderson

Project Officer (NCI): Dr. Andrew J. Peacock

Objectives: To study the distribution of haptoglobin types and the frequency of the Hp^1 allele in patients with carcinoma of the pancreas, in controls, and in patients with other neoplasms, and thereby determine if the haptoglobin genotype influences the development of pancreatic carcinoma.

Major Findings: A total of 2,312 cancer patients have now been typed for haptoglobin. Included in this total are 50 patients with documented carcinoma of the pancreas. Haptoglobin typings are also available on 288 controls, comprised of platelet and blood donors and University of Texas personnel and students. Pancreatic cancer patients manifest a high Hp^1 frequency (Hp^1= 0.459) compared with controls (Hp^1 = 0.384) or other cancer patients (Hp^1=0.390).

The type of pancreatic cancer seemingly has some effect since patients with adenocarcinoma have a markedly higher frequency (Hp^1= 0.476) than those with islet cell carcinoma or leiomyosarcoma (Hp^1= 0.357). Males develop adenocarcinoma of the pancreas 1.3 times more frequently and develop it at an earlier average age (56.9 years) than females (62.2 years). The Hp^1 frequency in males is also lower (Hp^1= 0.458 vs. 0.500). It is of further interest that the average age at diagnosis is correlated to some extent with haptoglobin types, in that Hp1-1 type individuals have an earlier diagnosis than Hp2-1 individuals, and the latter an earlier diagnosis than Hp2-2 individuals, with the average ages being younger in males than females. No such age trend or sex difference is evident for the total sample of patients with other cancers, or the few patients with islet cell carcinoma of the pancreas. The pancreatic cancer patients manifest a normal ABO and Rh blood type distributions so they appear internally consistent and representative of the general population. These results thus suggest that the Hp^1 allele is increased in pancreatic cancer, and in some way enhances the development of adenocarcinoma of the pancreas.

An added and totally unsuspected finding has been that six of the 50 patients had positive family histories of pancreatic cancer. This frequency is comparable to the familial occurrences of colon cancer and breast cancer observed at this institution. These familial occurrences were all among siblings of the patient (4 brothers and 4 sisters), and pertained to adenocarcinoma of the pancreas occurring independently of chronic pancreatitis. Islet cell carcinoma and pancreatic cancer developing in association with chronic pancreatitis have long been known to be familial, but adenocarcinoma occurring independently of chronic pancreatitis has only been previously reported in one family by MacDermott and Kramer (Gastroenterology 65: 137-139, 1973). The present study adds seven additional cases. No information is presently available to determine if these familial cases refer to pancreatic adenocarcinoma per se, to hereditary adenocarcinomatosis of which pancreatic cancer could be a part, or to some other hereditary syndrome.

Significance to Biomedical Research and the Program of the Institute: Cancer of the pancreas is an increasingly frequent form of disease. Very little is known of the etiology nor why the incidence is increasing. This work investigates some genetic factors that may be operative, and shows that some individuals are more susceptible than others. Knowledge of factors that lead to susceptibility (or resistance) would lead to more realistic assessment of carcinogenic hazards.

Proposed Course: This project is in its terminal year and will expire May 18, 1974.

Date Contract Initiated: May 19, 1971

Current Annual Level: $19,224

TEXAS, UNIVERSITY OF (M.D. Anderson Hospital and Tumor Institute)
(NO1-CP-43270)

Title: Non-Histone, DNA-Binding, Proteins from Normal Rat Liver and Chemically Induced Rat Hepatomas

Contractor's Project Director: Dr. Lubomir S. Hnilica

Project Officer (NCI): Dr. C. Wesley Dingman

Objectives: The objective of this project is the isolation and characterization of significant amounts of a tissue-specific group of non-histone chromosomal proteins from normal rat liver and from rat hepatomas.

Major Findings: A non-histone protein fraction of limited heterogeneity was isolated from rat liver and Novikoff hepatoma chromatin. In complexes with homologous DNA, this protein fraction is strongly antigenic, eliciting tissue-specific antibodies. Neoplastic transformation changes the antigenic tissue-specificity of these proteins to a new type, characteristic for cancerous tissues. In reconstitution experiments, the tissue-specificity of the non-histone protein-DNA complexes was determined by the tissue origin of the non-histone protein fraction and not by the origin of DNA. However, complexes of this non-histone protein fraction with DNA isolated from other animal species (heterologous) were not immunoreactive when challenged with antibodies against similar complexes with homologous DNA.

The tissue-specific fraction of chromosomal, non-histone proteins binds strongly to the DNA of the same species (homologous). As determined by affinity chromatography or sucrose density gradient centrifugation, unfractionated homologous (rat spleen) DNA can bind approximately 1% (w/w) of the tissue-specific, non-histone proteins from rat liver chromatin. Binding studies using DNA fractionated to Cot$<10^{-1}$, 10^{-1} to 200, and >200 showed that the tissue-specific, non-histone proteins from rat liver bind preferentially to the highly repetitive (Cot $<10^{-1}$) DNA.

Significance to Biomedical Research and the Program of the Institute: One of the major goals of the Carcinogenesis Program is the elucidation of the mechanism of action of known carcinogens. One hypothesis for malignant transformation is that carcinogens alter the regulation of genetic transcrption, either by a direct effect on particular genes or by an alteration of those proteins which function in regulation of gene transcription. The purpose of this contract is to fractionate and characterize, by present techniques, that set of proteins which are now thought to act as regulators of genetic function and then to determine whether or not members of this set are altered in amount or kind during the induction of hepatomas in the rat by chemicals. Knowledge of these biochemical processes would permit the study of ways to influence or otherwise modify significant steps in the malignant transformation process.

Proposed Course: The proteins, once isolated, are to be fractionated and examined for species which show qualitative or quantitative differences between normal liver and tumor. Such species will then be further characterized

as to their physical properties, enzymatic properties, and the specificity of their derivation from normal or malignant hepatic parenchymal cells.

Date Contract Initiated: June 29, 1972

Current Annual Level: $83,500

WEIZMANN INSTITUTE OF SCIENCE (NO1-CP-02217)

Title: Relationship of Polycyclic Metabolism to Carcinogenic Transformation

Contractor's Project Director: Dr. Leo Sachs

Project Officer (NCI): Dr. Harry Gelboin

Objectives: To clarify the role of polycyclic metabolism in carcinogenesis.

Major Findings: Finding show the chromosomal control of carcinogenesis and the level of hydroxylated enzymes in human epithelia cells and fibroblasts. These studies relate to the ability to transform human cells by chemical carcinogens.

Significance to Biomedical Research and the Program of the Institute: An understanding of these biochemical processes is necessary to understand carcinogenesis in humans.

Proposed Course: To further elucidate the mechanism of transformation and reversion of human cells.

Date Contract Initiated: May 26, 1970

Current Annual Level: $136,243

WEIZMANN INSTITUTE OF SCIENCE (NO1-CP-33220)

Title: Alteration in Translation of Genetic Messages Induced by Viruses and Carcinogens

Contractor's Project Director: Dr. Michel Revel

Project Officers (NCI): Dr. Dolph Hatfield
Dr. Harry Gelboin

Objectives: To determine the mechanism(s) of viral expression at the level of translation and how chemical carcinogens may affect this expression.

Major Findings: Messenger RNA for the SV40 major capsid protein (VP-1) has been selectively isolated from the host cell by using an oligo(dT)-cellulose column and hybridization to SV40 DNA. This mRNA has been translated in in

vitro protein synthesis systems and the product characterized as VP-1. The selective isolation of VP-1 mRNA provides the contractor with a means of determining when viral mRNAs appear and the cellular changes which occur in early and late expression.

Significance to Biomedical Research and the Program of the Institute: A better understanding of viral expression and the effect of chemical carcinogens on this process may lead to a mechanism of controlling viral expressions, and, therefore, provide a means of curing or preventing many forms of cancer.

Proposed Course: To investigate the interactions between the cell and virus which lead to the expression of early and late oncogenic viral mRNAs and to investigate the effect(s) of chemical carcinogens on this process.

Date Contract Initiated: June 1, 1973

Current Annual Level: $213,175

YALE UNIVERSITY (NO1-CP-43238)

Title: Selection and Propagation of Haploid and Partial Monosomic Cell Lines

Contractor's Project Director: Dr. Frank H. Ruddle

Project Officers (NCI): Dr. John Bader
Dr. Dolph Hatfield

Objectives: The overall objective is to generate a number of murine cell lines in vitro which are monosomic, partially monosomic, or haploid in their chromosomal composition. These lines will be particularly useful in the isolation of specific mutant cell lines which can be useful in a number of ways, including chemical carcinogenesis, viral carcinogenesis, and gene wapping.

Major Findings: As this is a new contract, no major findings can be reported at this time.

Significance to Biomedical Research and the Program of the Institute: The resolution of the nature of carcinogenesis requires an ability to manipulate biochemical pathways in mammalian cells. Selection of monosomite cells is essential to such manipulation, since diploid cells pose special continuing problems.

Proposed Course: Work will begin with the isolation of purified populations of spermatids and an attempt will be made to activate them with enucleated and nucleated proliferating tissue cultured cells.

Date Contract Initiated: December 18, 1973

Current Annual Level: $99,500

SUMMARY REPORT

COLON CANCER SEGMENT

July 1, 1973 through June 30, 1974

The Colon Cancer Segment has the aim of identifying the etiologic factors responsible for colonic cancer in man. It combines laboratory studies with epidemiological observations in order to determine the etiological factors leading to colon carcinogenesis and those involved in its control.

Most hypotheses interrelate diet and intestinal flora with the production or activation of carcinogens in the large bowel. Analyses by Biometry Branch, Field Studies and Statistics Program, DCCP, NCI, in collaboration with the former Director of the Colon Cancer Segment have correlated beef consumption with the incidence of colonic cancer. The program, which is now ending its third year, is beginning to come to fruition.

The Segment is sponsoring studies to define the relationship of diet to intestinal flora and that of the flora to multiple colonic polyps and cancer through a Field Studies and Statistics Contract with Virginia Polytechnic Institute (VPI) and with Carcinogenesis-sponsored studies at University of California (at Los Angeles [UCLA]) and the University of Missouri. The vast amount of data that has accumulated from the studies of the microflora in different populations is now undergoing statistical analyses. In the study of the Japanese-Hawaiian cohort by the Anaerobe Laboratory, VPI, 113 distinct types of microorganisms were detected among the 1147 isolates obtained from subjects in the study of the relationship of diet to the flora. The identification of organisms isolated from subjects diagnosed as polyp patients is still in progress. These investigators also observed that statistically significant differences occurred in the degradation of cholesterol in the intestinal tract of polyp patients when compared to normal controls. In polyp patients less bacterial conversion of cholesterol occurred and these individuals also excreted less total neutral steroids than did the controls.

At UCLA it was noted that differences in the flora were more marked between the two diet groups (Japanese vs Western) than between two different specimens from the same individual. Although it is too early to evaluate the data obtained by the University of Missouri, some preliminary observations are possible. This group is studying the changes in fecal chemistry and flora resulting from dietary changes. Subjects are examined while consuming their normal diet and when placed on a meat-free diet, on one with a high beef component, and following a return to their usual dietary habits. Considerable difference was noted in the numbers of different types of bacteria between subjects on the same diet as well as between samples from an individual on the same diet. In the absence of meat there was an increase in the total number of oxygen tolerant microorganisms. Their numbers decreased to the usual level when the subject was placed on a high meat diet. Evidence was found for the tryptophane metabolites, kynurenic sulfate and indoxyl sulfate, in the fecal samples and differences in free and total amino acids occurred when the diet was altered.

The American Health Foundation observed that germ-free rats were less prone to tumor formation than were conventional animals when 1,2-dimethylhydrazine (DMH) was injected by the subcutaneous route while N-methyl-N-nitro-N-nitroso-guanidine produced tumors equally in both germ-free and conventional animals. Animals injected with DMH produced more tumors when maintained on a high fat diet. This laboratory had confirmed earlier reports by others that persons on a high meat diet have a greater proportion of anaerobic than aerobic bacteria in their feces as well as increased concentration of bile acids and neutral steroids which are degraded to a greater extent than in the gut of individuals on a vegetarian diet. Fecal extracts from persons on a high meat diet showed greater β-glucuronidase activity than did those on a low-meat or non-meat diet.

At the NCI's Frederick Cancer Research Center, studies are performed on the metabolism of the intestinal bacteria isolated from patients with a history of adenomatous polyps in order to determine whether the intestinal microflora can produce carcinogens or co-carcinogens from such secretions as bile acids or food components as tryptophane. None of the tryptophane metabolites reported to be carcinogenic for the bladder have yet been isolated from cultures of organisms grown in the presence of this amino acid. Rapid *in vitro* and *in vivo* tests for mutagenicity are also under investigation for use as screening techniques. Thirty-six bile acids or derivatives were found to have no mutagenic effect.

At the Memorial Hospital, the kinetics of intestinal epithelial cell development and the changes in enzyme activity during maturation of both normal and tumor cells are being studied. Two phases in the development of proliferative lesions have been described. Differences were found in the activity of cell enzymes concerned with DNA synthesis and in cell maturation. A routine cytological examination, using material obtained either by biopsy or by washing, has been developed and appears to be promising for early diagnosis of large bowel tumors.

Epidemiological studies are in the early stages of data collection in a two part investigation of colonic cancer among blacks. In a coordinated program with the Kaiser Foundation Research Institute and at the Medical College of Georgia, a diet questionnaire has been prepared and patients and control subjects are being interrogated. The study is designed to correlate diet and diet changes with the incidence of colonic cancer among southern blacks and blacks who have migrated to the San Francisco Bay Area and who are in a transition period toward a population at higher risk.

During the year the Segment's support staff has been reduced to one full-time and one part-time member. As a result, expansion of the program to determine the etiology and prevention of this most prevalent cancer cannot be significant. However, a modest effort will be made to exploit the VPI observation that differences in cholesterol metabolism occur between normal subjects and those with a history of intestinal polyps.

CONTRACT NARRATIVES

COLON CANCER SEGMENT

July 1, 1973 through June 30, 1974

AMERICAN HEALTH FOUNDATION (NO1-CP-12310)

Title: Experimental Large Bowel Carcinogenesis

Contractor's Project Directors: Dr. John H. Weisburger
Dr. Ernst L. Wynder
Dr. Bandaru Reddy

Project Officer (NCI): Dr. Sidney J. Silverman

Objectives: To determine the mechanisms by which the high meat protein and fat diet, known from epidemiologic data to be associated with a high colon cancer risk, is translated into specific carcinogenic, cocarcinogenic and/or promoting factors which affect the large bowel; to establish suitable animal models of colon carcinogenesis in bacteriologically defined and controlled systems; to determine the effect of dietary factors as modifiers of colon carcinogenesis; to determine the carcinogenicity of selected bile metabolites; to carry out parallel studies in animal and man relating the role of diet, in general, and acid and neutral steroids and other endogenous metabolites, in particular, in colon carcinogenesis; and to isolate pure strains of the major groups of intestinal microflora from high and low risk populations, and study their enzymatic capability to degrade acid and neutral steroids into potentially carcinogenic or cocarcinogenic compounds.

Major Findings: Rats fed a high fat diet and injected with the carcinogen 1,2-dimethylhydrazine exhibited greater yield of colon cancer than rats fed lower fat diets. Animals on the high fat diet also exhibited increased bile acid and neutral sterol excretion, and also an increased microbial metabolism of these compounds. In studies on the intervention of the gut microflora in the mechanism of action of dimethylhydrazine, it was shown that germ-free rats had a lower yet definite incidence of colon cancers than conventional control rats. Also, the tumors seen appeared later in the germ-free rats compared to controls. Thus, the microflora plays a modifying role in the induction of colon cancer with this agent which, by current concepts, requires metabolic activation. Further evidence on this point was derived from the fact that intrarectal injection of N-methyl-N^1-nitro-N-nitrosoguanidine (MNNG), an agent which does not require enzymic but only chemical hydrolytic activation, led to an identically high incidence of colon cancer with similar latent periods in germ-free and in conventional rats.

Comprehensive studies have begun on the possible carcinogenicity or co-carcinogenicity of select bile acids, neutral steroids, and their key metabolites. It has been found that administration by the intrarectal route, pioneered in this laboratory, of two bile acids, taurodeoxycholic acid and

lithocholic acid, significantly increased the colon tumor yield induced by a single intrarectal dose of MNNG given ten days prior to initiating the promoting treatment with the bile acids. This is the first time that such a promoting activity has been discovered for bile acids or their metabolites. Others have postulated the possible involvement of polycyclic aromatic hydrocarbons in colon carcinogenesis. In a preliminary series of tests it has been observed that, in a number of such chemicals, the intrarectal instillation of 3-methylcholanthrene has resulted in a number of colon tumors.

Americans consuming a mixed Western diet excreted higher levels of bile acids and neutral sterols compared to low risk populations of Japanese, Chinese, Americans, Seventh Day Adventists, and vegetarians. A controlled study involved the same individuals on two distinct diets, high and low risk for colon cancer. On a mixed Western diet, including meat, their stools contained increased levels of bile acids and neutral sterols as well as β-glucuronidase activity compared to when they were on a non-meat diet. Certain of these metabolites and enzymes appear to be useful "markers" which distinguish low and high risk populations for colon cancer. The development of such key marker systems will be extended to additional, hopefully, crucial markers to delineate sensitive populations in animal models and applicable to man.

Significance to Biomedical Research and the Program of the Institute: Colon cancer constitutes one of the major types of cancer in the United States and in the Western World. The etiology of colon cancer, and factors and mechanisms related thereto, are unknown. The current long-term effort is part of a key program to link animal experimentation and the study of human cancer causation.

The information and new developments provided by this contract are designed to yield ultimately an understanding of the contribution of exogenous and endogenous factors such as diets, intestinal microflora, and bile constituents to the etiology of colon cancer in man. Evaluation of the main elements in this complex puzzle may eventuate in the formulation of a rationale and sound set of preventive approaches.

Proposed Course: (1) To study DMH carcinogenesis and MNNG carcinogenesis in germ-free and defined flora rats; (2) to continue studying fecal chemistry and microflora in populations of epidemiologic interest with regard to colon cancer, and in patients with colon cancer and with polyps; (3) to study the effect of high meat and high animal fat diet, in relation to appropriate controls, on the chemical components and microflora of feces; (4) to investigate the effect of different meat diets on chemical components of feces in germ-free and control rats; (5) to test the carcinogenicity of bile metabolites in the mouse skin induction-promotion system, as well as in germ-free and conventional rats by intrarectal infusion; (6) to study the ability of pure strains of fecal microflora, isolated from groups of populations on different diets, to degrade or metabolize acid and neutral steroids and other bioactive compounds into potentially carcinogenic compounds; and (7) to develop information on the metabolism of nitrosomyoglobin and other nitrosated chemicals in germ-free and conventional rats.

Date Contract Initiated: June 30, 1971

Current Annual Level: $382,567

CALIFORNIA, UNIVERSITY OF (at Los Angeles) (NO1-CP-33380)

Title: Fecal Flora Studies

Contractor's Project Director: Dr. Sydney Finegold

Project Officer (NCI): Dr. Sidney Silverman

Objectives: To determine whether differences exist in the intestinal flora of individuals subsisting on Western and Japanese diets and between individuals with intestinal polyps and case controls.

Major Findings: Persons on the Japanese diet had a greater prevalence and variety of lactobacilli and higher counts of Streptococcus bovis than those on the Western diet.

Extremely oxygen-sensitive anaerobes and spirochetes were more common in subjects on the traditional diet. Contrary to our earlier results, there was no difference in Fusobacterium counts related to diet. Unidentifiable Bacteroides species were more common on the Japanese diet. Peptostreptococcus was distinctly more prevalent in people eating Japanese food, but certain other anaerobic cocci (Acidaminococcus, Veillonella and Peptococcus magnus) were seen more in relation to the Western diet. Megasphaera was seen only in two subjects on a Japanese diet and Sarcina only in one subject on the Western diet. Gram-positive non-sporulating anaerobic bacilli were prevalent in both groups, but Bifidobacterium adolescentis and B. infantis were more common with the Western diet and Eubacterium contortum, E. lentum and E. rectale were more common with the Japanese diet.

Second specimens were obtained after a 2 to 19 week interval from 7 subjects, the bacteriologists again being unaware of this. The nature and extent of the differences between the two diet groups was more pronounced than that between duplicate specimens from the same individuals. If these differences in fecal flora can be verified as related to diet by additional studies, this may provide important leads towards determination of specific etiologic factors in bowel cancer. The data is being analyzed statistically at the present time.

The study also provided valuable information on the normal human fecal flora and on optimum media and techniques for defining this flora accurately and expeditiously. B medium was found to be excellent for recovery of Bifidobacterium as was Fusobacterium medium for this genus. Clostrisel agar and blood agar used with heated dilutions of specimens were less satisfactory for recovery of clostridia than egg yolk agar with heated dilutions and rifampin agar. Egg yolk-Nagler plates and Veillonella neomycin agar were also useful for selected species of clostridia. LBS medium, the best single

medium for lactobacilli, was found to be less than satisfactory in that a number of these organisms were recovered from other media and not from LBS. Desoxycholate agar proved to be better than nitrogen deficient agar for recovery of Klebsiella. Sabouraud's agar gave better yields of both yeasts and molds than did molybdate agar.

Comparison of the fecal flora of subjects with multiple colonic polyps with that of matched controls with demonstrated (by colonoscopy) lack of polyps is presently underway. Seventeen subjects of each type have been studied so far, but the bacteriological data is not yet complete enough to permit analysis. A total of 25 patients and 25 controls will be studied in a one-year period.

Significance to Biomedical Research and the Program of the Institute: To date, a great deal has been learned of the nature of the normal human fecal flora and of optimum ways of defining it. This information will be useful in many ways other than its possible relationship to colon cancer. A number of striking differences in fecal flora apparently related to diet have been noted. If statistical analysis confirms these differences, this may provide important clues as to organisms which may produce carcinogens or in other ways be related to bowel cancer. Other organisms may also be shown to have a protective role. Similar differences in a still higher risk population (patients with multiple adenomatous polyps) would reinforce the significance of the results.

Proposed Course: The present studies of fecal bacterial flora in subjects on different diets and of persons predisposed to colon cancer, as well as controls, will be continued. Comparison of two Seventh Day Adventist populations (one, lifetime vegetarian and the other, non-vegetarian) is proposed. All organisms will be identified and quantitated and the data analyzed to determine whether significant differences exist.

Date Contract Initiated: June 30, 1971

Current Annual Level: $147,308

GEORGIA, MEDICAL COLLEGE OF (NO1-CP-43282)

Title: Epidemiologic Studies of Colon Cancer Among Blacks

Contractor's Project Director: Dr. Warren H. Gullen

Project Officer (NCI): Dr. Ann E. Kaplan

Objectives: Determination of relationship of diet (especially animal protein, fat, and refined carbohydrate) to the occurrence of cancer of the colon.

Major Findings: Since the developmental phase of this study has just been completed and the data collection is beginning, no major findings can be reported at this time.

Significance to Biomedical Research and the Program of the Institute: Successive cancer surveys by NCI have shown that the incidence rate for colon cancer among Southerners has been, and remains, lower than the rate for Northerners. Indeed, between the last two national NCI cancer surveys, the rate for Northern blacks has risen to equal that among Northern whites while the rate for Southern blacks remains lower than that for Southern whites, which in turn is lower than that for Northerners. This observation, along with the results of studies conducted by NCI investigators and others, tends to implicate diet as an etiological factor. Attempts to confirm the etiological role of diet in studies of relatively high-colon-cancer-rate populations have, to date, been unsuccessful. Therefore, this study is being executed among a population with a relatively low colon cancer incidence in order to derive more definitive data bearing on the hypotheses.

Proposed Course: To conduct, in the next two and one-half years, a retrospective case-control study of colon cancer in several Atlanta area hospitals, with investigation of differences in dietary history between persons with and without colon cancer.

Date Contract Initiated: June 30, 1972

Current Annual Level: $63,315

KAISER FOUNDATION RESEARCH INSTITUTE (NO1-CP-33215)

Title: Epidemiologic Study of Colon Cancer Among Blacks

Contractor's Project Director: Dr. Gary Friedman

Project Officer (NCI): Dr. Ann E. Kaplan

Objectives: To search for dietary factors which may be associated with cancer of the large bowel and to identify specific dietary components which have implications for prevention of the disease.

Major Findings: The first year of data gathering is in progress, and no findings are available at this time.

Significance to Biomedical Research and the Program of the Institute: Research on Japanese migrants to Hawaii has implicated dietary factors in the etiology of cancer of the large bowel. The present study is one of the few undertaken to explore the association between diet and the disease in Americans living in the continental United States. If the Hawaiian findings are confirmed, the results will permit greater specificity in the laboratory testing of chemical and bacteriologic agents in the disease process. Preventive dietary modifications may also be suggested.

Proposed Course: Cases of cancer of the large bowel among a group of relatively low risk Blacks are being identified in hospitals in five San Francisco Bay Area counties. The estimated 195 cases are to be compared with age-sex-

race-matched controls (three per case) on diet history and relevant demographic information.

Date Contract Initiated: December 1, 1972

Current Annual Level: $26,415

NCI, FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc.) (CN-23294)

Project 7

Title: Laboratory Studies of Bowel Cancer Etiology

Contractor's Project Manager: Dr. Milton Slein

Project Monitor (NCI): Dr. Sidney Silverman

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CP-22041)

Title: Regulatory Control of Cell Proliferation in Colonic Tissue (in Familial Polyposis)

Contractor's Project Director: Dr. Martin Lipkin

Project Officer (NCI): Dr. Lionel Poirier

Objectives: To study the sequence of events and regulatory control abnormalities leading to uncontrolled proliferative activity and the initiation of carcinogenesis in this disease inherited as a dominant gene defect.

Major Findings: (1) Phase 1 Proliferative Lesion in Colon Cells in Familial Polyposis - Flat colonic mucosa epithelial cells that have migrated to the mucosal surface continue to incorporate thymidine into DNA. The overall kinetics of cell proliferation are normal (birth rate = extrusion rate) without a net accumulation of cells. These cells do not readily move through G_2 into mitosis. The defect is present in flat mucosa of patients with polyps and relatives who have not developed polyps.

(2) Phase 2 Proliferative Lesion in Colon Cells in Familial Polyposis - Cells that fail to repress DNA synthesis also develop additional properties that enable them to be retained in colonic mucosa in increasing numbers. The overall kinetics of cell proliferation are abnormal (birth rate >extrusion rate) and net accumulation of cells begins. This abnormal proliferative phase is present in adenomatous polyps of family members who develop growths. Findings of Phase 1 and Phase 2 lesions in familial polyposis, in non-hereditary colonic neoplasms in man, and in mice after chemical carcinogen

treatment suggest that these lesions are major changes on a common pathway leading to malignant transformation.

(3) Persistent Thymidine Kinase Activity in Neoplastic Growths in Familial Polyposis - As normal colonic epithelial cells migrate through the middle third (transitional zone) of colonic crypts, thymidylate synthetase (TS) activity decreases earlier than thymidine kinase (TK) activity, and TS appears to have an earlier regulatory role in the normal cessation of DNA synthesis observed. But, as cells develop the characteristics of the Phase 2 proliferative lesion, TK activity remains high in surface cells, the profile of enzymes activity resembles middle third (transitional) cells, and continued activity of the TK salvage pathway contributes to the persistent thymidine incorporation and to the continued DNA synthesis observed.

(4) Clinical Screening of Severity of Precancerous Change - With biopsies and cytological washings from colonic mucosa, Phase 1 and 2 proliferative abnormalities and increasing degrees of cellular atypia can be screened in patients with familial polyposis. The routine screening procedure has resulted in cytological evidence of early malignant change in colonic mucosa of one family member and appropriate surgical intervention.

Significance to Biomedical Research and the Program of the Institute: Although environment influences the development of colon cancer, hereditary factors also have a major influence on whether individuals will develop the various transitional proliferative changes observed and malignancy. Current findings indicate that the proliferative malfunctions observed in familial polyposis also develop in individuals with isolated non-hereditary colonic lesions and in mice after the carcinogen 1,2-dimethylhydrazine. Findings suggest that these are major changes on a common pathway leading to carcinoma, and that environmental and chemical carcinogens induce the changes found leading to colon cancer in man. In familial polyposis, the abnormalities develop more rapidly and more widespread in the colon than in non-hereditary disease, making available a human model for study of the mode of interaction of carcinogenic factors with colon cells in this "experiment in nature."

Proposed Course: The proliferation kinetics of colonic epithelial cells in familial polyposis will be studied with particular reference to the G_2 phase leading to mitoses and rate of loss and turnover of polyposis cells. The metabolic pathways leading to uncontrolled proliferative activity will be studied with further analysis of reasons for persistent thymidine kinase activity and its role in the early molecular regulatory anomaly observed. Attempts will be made to develop methods of propagation of colonic tissue in various stages of neoplastic transformation. The current clinical screening program will be continued to detect and develop new methods for detection of premalignant changes in colonic cells of patients with familial polyposis, and additional approaches to prediction of susceptibility to cancer in man will be studied.

Date Contract Initiated: October 15, 1971

Current Annual Level: $95,000

MISSOURI, UNIVERSITY OF (NO1-CP-33335)

Title: Effect of a High Meat Diet on the Bacterial Flora and Chemical Components of Feces

Contractor's Project Director: Dr. David Hentges

Project Officer (NCI): Dr. Sidney Silverman

Objectives: To examine the effect of a high meat diet on the composition of the intestinal flora and on chemical components of the feces.

Major Findings: No definite conclusions can be drawn from the data that are currently available. A few preliminary observations can be made, however. Considerable variation in the numbers of different types of bacteria occurs from individual to individual and, within the same individual, from sample to sample even on the same diet. In assessing the effects of dietary change on flora composition, analyses are made in terms of each individual as well as the entire group. Despite the variations, there is some indication that the total number of aerobic and facultative organisms isolated from the volunteers increases during the meatless diet and then decreases to normal levels again during the high meat diet. The change from a meatless to a high meat diet also causes a simplification of the composition of the anaerobic flora so that fewer numbers of species are isolated. On the high meat diet, counts of Clostridium, Bifidobacterium, and Eubacterium are lower and Bacteroides and Propionibacterium counts are higher than when the volunteers are on a meatless diet.

In the analysis of feces of individuals for the presence of tryptophan metabolites, some evidence has been obtained that free, kynurenic and indoxyl sulfate are present. The feces of volunteers on a normal diet contains two to three times more combined, soluble amino acids, and about ten times more total amino acids than free amino acids. The concentration of free soluble amino acids in the feces is slightly greater when individuals are on a high meat diet than when on either a normal or a meatless diet.

Significance to Biomedical Research and the Program of the Institute: Epidemiological evidence correlates high meat consumption with the higher incidence of colonic cancer in western countries. Evidence that such a diet causes significant changes in the bacteriology of the intestinal tract, as well as in the chemical composition of intestinal contents, should suggest what factors (products of tryptophane, amino acid, or steroid metabolism) may be of etiologic importance.

Proposed Course: Volunteers will be maintained for 16 weeks on a dietary regimen consisting of a "normal" diet, a meatless diet, a high meat diet, and again a "normal" diet--each dietary period lasting four weeks. During the fourth week, three fecal specimens will be collected from each volunteer and assayed for bacterial composition and content of proximates, minerals, tryptophane metabolites, amino acids and other nitrogenous compounds, fatty acids, and neutral and acid steroids.

Date Contract Initiated: June 5, 1973

Current Annual Level: $240,648

OMNI RESEARCH INC. (NO1-CP-33244)

Title: Preparation of Bile Acids and Their Derivatives

Contractor's Project Director: Dr. Owen H. Wheeler

Project Officer (NCI): Dr. Sidney J. Silverman

Objectives: To obtain a series of bile acids with their derivatives for use in studies of the metabolism of anaerobic bacteria found in the large bowel.

Major Findings: A series of hydroxy, keto and hydroxyketo cholanic acids are being prepared for metabolic studies, using lithocholic, desoxycholic, chenodesoxycholic and cholic acids as starting materials. The keto acids are made by oxidation of the bile acids or an ester with chromic acid. The selectivity of the various hydroxy groups (7>12>3) towards chromic acids is also used to prepare the hydroxyketo compounds. The 3-position can also be selectively protected as the succinoxy derivative. Oppenauer oxidation attacks the 3-position preferentially. The 3β- and 12β-hydroxy derivatives can be prepared by catalytic reduction of the corresponding ketoacids. However, the 3β-derivatives are best prepared by reacting the 3α-tosylates with potassium acetate in dimethylformamide.

Significance to Biomedical Research and the Program of the Institute: This contract will permit more detailed studies of metabolic pathways used by anaerobes than has been possible previously.

Proposed Course: It is expected that all compounds will be delivered this fiscal year.

Date Contract Initiated: April 2, 1973

Current Annual Level: $12,700

OXFORD, UNIVERSITY OF (NO1-CP-23215)

Title: Study of the Relationship in Individuals between Colon Cancer and Epidemiologically Linked Diseases

Contractor's Project Director: Dr. John Baldwin

Project Officer (NCI): Dr. Sidney Silverman

Objectives: To investigate hypotheses that certain diseases (such as coronary heart disease) are caused by the same factors as specified bowel tumors, and will, therefore, be linked in individuals in a general population more frequently than would be expected by chance.

Major Findings: Analysis of the geographic distribution of colon cancer in England has just been completed and the report is being prepared.

Significance to Biomedical Research and the Program of the Institute: The association of colon cancer and other diseases in populations has been the subject of much discussion. Determination of the links in individuals would provide invaluable clues to etiology and, as well, provide markers of high risk for detection programs. While this kind of information is sought from U. S. sources, all are small and/or highly selective populations, and the data are not readily accessible to say the least. Major segments of later parts of the program will be based on these epidemiological findings.

Proposed Course: Existing computer programs will determine which diseases are associated with bowel tumors by determining which preceded or succeeded bowel tumors more often than would have been expected from the incidence in the general population of the region. Statistically significant associations will be subjected to case review to verify diagnoses and to rule out artifactual associations.

Date Contract Initiated: April 9, 1972

Current Annual Level: $12,000

SUMMARY REPORT

INFORMATION AND RESOURCES SEGMENT

July 1, 1973 through June 30, 1974

The Information and Resources Segment (I&RS) has three major areas of responsibility within the Carcinogenesis contract program. These are the broad general categories of animal, chemical, and information resources. Within these categories, the Segment responds to several particular types of needs. The first of these is to provide support to other segments with resource contracts which are essential for the continued functioning of that segment's activities but for which no particular expertise is available within that segment's working group. The second area of need lies in the anticipation, planning and provision for services which meet the resource needs of the total Carcinogenesis Program as distinct from the needs of specific segments.

An advisory group which includes five members of the scientific community external to NCI works with the Segment on a continuing basis. Since such a working group contains a limited range of expertise, it is necessary for us to draw on other members of the scientific community on an ad hoc basis to assist in the evaluation of individual proposals, in the conduct of site visits, and in the long-range planning of program resource needs. Such a program of involvement of scientists from the community as a whole is central to the operation of the Segment and, in fact, in this fiscal year fifty-one persons have been utilized on such an ad hoc basis, forty-six of them being from outside of the NCI.

It is projected that the Segment's activities at the end of this fiscal year will include 22 contracts and a total budget of approximately two million dollars. Slightly more than half of these funds will be expended in the area of information services; the greater part of the remainder will be devoted to chemistry resources with a somewhat lower level of activity in the animal resources area.

Chemical Resource Activities:

For the past two years a carcinogen standard reference compound bank has been in existence. The major source of the reference samples has been through a contract with Starks Associates, Inc. The contractor is acquiring, purifying, and characterizing specified compounds and repackaging them in smaller quantities for storage and distribution to investigators in the cancer research community. Over 60 such materials are currently available and more are being added on a continuing basis. The reference bank is now located at the NCI's Frederick Cancer Research Center (FCRC) (Litton Bionetics, Inc.) and will become part of the chemical repository that is being developed there. In addition to the existing reference bank, which will grow considerably, the repository will be responsible for the procurement and distribution of chemicals to be used in the bioassay program and in other areas of the program as need is demonstrated. It is anticipated that the repository will be in full operation in the coming fiscal year.

The IIT Research Institute provides support in meeting a specific resource need of the Lung Cancer Program. This group provides highly purified and characterized powders and suspensions for use in intratracheal instillation studies. The suspensions are composed of carcinogenic materials (or their non-carcinogenic analogs) in aqueous systems, are of high purity, and have known size distributions. The powders are prepared from carbon and a variety of metal oxides and have defined size distribution, shape, surface area, and porosity. The powders are provided to investigators either alone or after coating with benzo[a]pyrene. An additional program was initiated this year with the IIT Research Institute to study the stability of benzo[a]-pyrene when adsorbed on the surface of these particles. This study, which has now demonstrated the stability of the coated particles, also brought to our attention the fact that the method sometimes used to destroy benz[a]pyrene (exposure to ultraviolet radiation) is not effective for the solid material.

The collaborative program with Ash Stevens, Inc.continues to provide interested investigators with purine and pyrimidine nucleotides specifically tailored to function as selective irreversible inhibitors of nuclear RNAse. This aspect of the program is attempting the development of a selective drug probe enabling investigators to control the destruction of messenger RNA in the cell nucleus. In addition, a number of these products have been shown to function as inhibitors of the reverse transcriptase of oncogenic viruses and leukemia cells.

Contract programs in the field of nitrosamines and cancer have been expanded in the past year. A feasibility study to identify human populations at risk due to exposure to N-nitroso compounds was implemented this year with the Mt. Sinai School of Medicine. While there is ample evidence that nitrosamines are carcinogens in all species of animals that have been tested, there is no evidence for such an effect in humans. It is hoped that this study will indicate whether or not such a study could be conducted with reasonable hope of obtaining a conducive result. Two other projects are to be initiated before the end of the fiscal year. One is on the development and application of methods for the determination of N-nitroso compounds and their precursors in the environment. The other is for the synthesis of N-nitroso compounds which are labeled either with radioactive or stable isotopes.

Finally a task has been established at the FCRC to establish methodology and guidelines for the safe-handling of carcinogenic chemicals including safe methods of disposal.

Information Resource Activities:

Activities of the Segment in this area are designed to meet a variety of information needs generated by the scientist or administrator working in carcinogenesis research.

A continuing need exists to make more easily available, on a timely basis, the relevant information being generated in those disciplines which impact on the Carcinogenesis Program Area. This requirement is being met in part

by activities such as the journal, Carcinogenesis Abstracts, which is produced under contract with the Franklin Institute Research Laboratories. The magnitude of this activity is increasing continuously as the overall level of activity in cancer-related research rises.

Information which may offer leads as to the carcinogenic potential of specific molecules or classes of molecules is an ever present need in our program area. This results in a requirement for scanning of the pertinent literature to collect references to any long-term testing activities which appear and the extraction of the data into a uniform format. This activity is being carried out on a continuing basis under a contract with Tracor/JITCO, Inc. and results in the production of a series of published volumes entitled Survey of Compounds Which Have Been Tested for Carcinogenic Activity PHS-149. During the past year this series has been brought up-to-date and volumes now in press will result in complete coverage of the literature through 1973.

To make the information in these volumes more readily accessible, cumulative indexes have been prepared which permit entry from a variety of starting points. In addition to the usual parameters, these indexes include CAS registry numbers, formal nomenclature, synonymns, molecular formulas, and Wiswesser Line Notation (WLN). The line notation indexes, together with a cumulative permuted index, have been generated in collaboration with scientists at FCRC and the Institute for Scientific Information. These indexes are being converted to a machine readable form during this year so that searches can be conducted in a more timely fashion. In addition, it should be noted that the addition of WLN to the files permits limited substructure searching; while the addition of CAS registry numbers and formal CAS nomenclature increases the capability of linking our files with a variety of other data bases currently available.

In an attempt to maintain a state of current awareness of the substances which are undergoing long-term testing throughout the world, partial support is being provided to an activity being conducted by the International Agency for Research on Cancer (IARC). This has resulted in the establishment of an international registry of compounds undergoing long-term toxicity testing. The first registry listings became available during this contract year. Therefore, it may be possible to extend the capability of bioassay testing around the world by reducing duplication of effort.

The growing concern with our abilities to predict the carcinogenic potential of molecules based on reports of other biologic effects has caused the Segment to furnish partial support for the Environmental Mutagen Information Center (EMIC) located at the AEC - Oak Ridge National Laboratory. This program functions in the mutagenesis area in a fashion similar to our literature-related activities in the carcinogenesis area. Elements of chemical-mutagenesis information are being extracted into the EMIC data base in a manner which permits easy linkage to the index portions of PHS-149.

In an attempt to develop mechanisms which will assist scientists in making decisions as to which chemicals should receive priority in our testing or

investigational programs to best utilize the limited resources available, there is underway in collaboration with the Stanford Research Institute (SRI) a developmental program having as its objective the collection, analysis, and systematization of information on the use, production, and extent of human exposure for chemicals which have significant contact with man. The SRI investigators are now in the process of refining their ability to identify and quantitate specific population exposure levels.

In collaboration with the IARC a monograph series which provides a balanced evaluation of the available data on individual chemicals as to their carcinogenic risk to man is in production. Volumes 1 through 4 of this series are now available and two additional volumes are in press.

In January 1974 the I&RS sponsored a conference on Data Dissemination subtitled "Interrelationships between the Carcinogenesis Program and the Concepts, Techniques, and Problems of Data Dissemination." Participants, including information, physical, and social scientists as well as cancer researchers, were drawn from NCI, other government agencies, universities, and private and foreign organizations.

Five working groups were established to address the following topics: qualification of research data; scientific acceptability of data dissemination; economics and management of data dissemination; legal, legislative, and public affairs questions; and the impacts of a program of data dissemination.

The recommendations from the conference were for the development of a feasibility study to test the utility and effectiveness of dissemination of data from projects within the Carcinogenesis Program. Such a study might ultimately lead to the establishment of a data analyses center.

Finally this year a new approach to handling information needs is undergoing a trial. A basic ordering agreement has been established with the Franklin Institute Research Laboratories. This agreement has made it possible to respond rapidly to the information needs of the Carcinogenesis Program. It has been set up to handle requests ranging from simple literature searches to critical reviews of a given topic. To date the need for this project has been well demonstrated and the number of requests have been increasing rapidly.

Animal Resources:

The Segment has some responsibility for the development of new animal models for cancer research. The wild European hamster (Cricetus cricetus L.) has been observed to develop bronchogenic epidermoid cancer similar to that seen in man. Attempts by the Medizinische Hochschule to domesticate and breed these animals under laboratory conditions has been quite successful. The colony, which numbers approximately 500 animals, is now in the F_4 generation with laboratory-raised animals having lost the aggressiveness and poor-breeding characteristics of wild-caught animals. Studies with several carcinogens have confirmed their susceptibility to epidermoid lung

cancer. In addition, an excellent, well-illustrated anatomical atlas of the European Hamster has been produced and is now in review. A colony of marmosets is being maintained temporarily by the Segment at AEC - Oak Ridge Associated Universities as a result of expressed interest in the animals as models for the study of colon cancer by both the Biology and Immunology Segment and the Colon Cancer Segment. Marmosets, which are small primates, have the potential of being useful animals for the study of carcinogenesis, particularly in the study of colon cancer since one species spontaneously develops the disease.

In the past year, the Biological Models Segment expressed a need for aged male rats for their prostate cancer research program and has asked for assistance in developing a source.

Although the Animal Farm at FCRC is now supplying the entire animal needs for the Bioassay Program, a second source of the commonly used rodents, the Fisher 344 rat and the $B6C3F_1$ mouse was felt to be necessary. A RFP to accomplish this was issued this fiscal year. The project will eventually produce (by the end of the second contract year) numbers of animals roughly equal to one-fifth or one-sixth of the FCRC effort.

A contract with the Massachusetts General Hospital, in collaboration with the Angell Memorial Animal Hospital will provide monographs on tumors of various organs in the dog and cat with a comparison of tumors of man. The first two monographs on the respiratory system and thyroid gland are in the process of being reviewed.

The Segment is responsible for providing information on animals and animal care to the Carcinogenesis Program. Current standards of animal care are not addressed to the long-term holding of animals or many host-environment considerations. In an effort to develop such guidelines the Segment sponsored a workshop on this in April 1973. The Institute of Laboratory Animal Resources (NAS/NRC) will publish the proceedings and develop standards for long-term animal care under contract with the Segment.

Two new contracts will be initiated in the near future to provide pathology support to the program, thereby improving program resources for both routine and experimental pathology.

CONTRACT NARRATIVES

INFORMATION AND RESOURCES SEGMENT

July 1, 1973 through June 30, 1974

AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge Associated Universities (Y01-CP-40013)

Title: Spontaneous Colonic Cancer in the Marmoset

Contractor's Project Director: Dr. Nazareth Gengozian

Project Officer (NCI): Dr. John A. Cooper II

Objectives: To maintain an established marmoset colony to observe the incidence of a spontaneously occurring adenocarcinoma of the colon.

Major Findings: In a previous report we summarized our finding that cottontop marmosets (*S. oedipus*) in our colony were dying with a primary carcinoma of the cecum and ascending colon that metastasized early to regional and distant mesenteric lymph nodes. This cancer has not occurred in the white-lipped marmoset (*S. fuscicollis* ssp.). We described seven individual cases gleaned from 49 necropsies where the malignancy of locally invasive mucin-producing mucosal cells was proven by lymph nodes metastases that were often more detectable than the small primary sites. An eighth case was excluded because metastases could not be demonstrated. No suspicious cases were found in approximately 500 necropsies of *S. fuscicollis*. In the succeeding 4 months of this contract we have husbanded this colony to determine whether this experience was going to continue. During this time 23 adults (older than 6 months) among 410 *S. fuscicollis* and two of 48 *S. oedipus* died and were necropsied. We report now an additional case of primary carcinoma of the cecum with local invasion of the cecum and numerous lymph nodes metastases in one of the two dead cottontops and none in the other species. Typically this animal had been in the colony for about 4 years, had been caught in the wild and suffered a wasting illness associated terminally with diarrhea and an enlarging abdomen. Death was secondary to ileal obstruction caused by a local lymphatic invasion of the ileo-cecal valve ring and terminal ileum. This invasive cancerous process was accompanied by enormous smooth muscle hypertrophy. Nothing in this or the other animals' histories give us any clues as to what the etiologic agent might be. The fact that the cancer occurred in one animal that was colony-bred seems to suggest that the agent can be acquired in our laboratory. Of particular interest of the moment is the local inflammatory process that is rich in lymphocytes, plasma and grape cells and histocytes rich in muco-polysaccharides.

Significance to Biomedical Research and the Program of the Institute: Colonic cancer in man is second only to lung cancer as a cause of death related to a malignancy. Our findings of an inordinately high incidence of colon cancer in one species of marmoset (*S. oedipus*) and the apparent absence of a similar malignancy in another species (*S. fuscicollis*) appears to provide us with two closely related animal systems which may have different susceptibilities to

known (or unknown) colon-carcinogens. Although colon cancers can be induced in other animal models, the high incidence of spontaneous cancer in the marmoset suggest that data obtained through future carcinogenic studies in this species may be more meaningfully extrapolated to man. The relatively long life span of the marmoset (10-17 years) compared to other animal models also comes closest to realizing the natural biological factors of aging in man which may play a significant role in the development of this disease. Further investigation, therefore, of the marmoset in problems related to this cancer seems extremely desirable, particularly in view of the twinning and immunologic tolerance phenomenon characteristic of this species which could lend itself to a controlled immunotherapeutic approach to cancer in a primate system.

Proposed Course: The project will continue as a program resource until the end of the current support period. By that time it is anticipated that evaluation of the model system by other segments in our program will have progressed to the point that a decision can be made as to the requirement for long-term support.

Date Contract Initiated: July 1, 1973

Current Annual Level: $23,000 (6 months)

AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory) (Y01-CP-20203)

Title: Environmental Mutagen Information Center (EMIC)

Contractor's Project Director: Dr. J. S. Wassom

Project Officer (NCI): Dr. Sidney Siegel

Objectives: The mission of the Environmental Mutagen Information Center (EMIC) is to collect, organize, and disseminate chemical mutagenesis information. Information contained in this unique file may either report on the testing of chemicals in one of the many available mutagenic assay systems or contain pertinent data which can be used in understanding the known or suspected mutagenic activity of environmental chemical agents. The information collected for this data base is to be processed and disseminated to the scientific community.

Major Findings: Approximately 5,000 references were added to the EMIC data base during FY 1974. This figure brings the total number of references on file to approximately 14,000. All of these citations have been indexed with Agent/Organism keywords and Chemical Abstracts Registry Numbers. From these indexes, a listing of chemicals based on frequency in the EMIC Registry was prepared. Results from this list indicated that 180 compounds had been evaluated at least 10 times for mutagenic effects. The most frequently tested compound was the known carcinogen-mutagen, N-methyl-N'-nitro-N-nitrosoguanidine, which was cited 787 times. Correlations with information available in PHS 149 was also made using this listing on all the 1,500 compounds present in the EMIC Registry.

Significance to Biomedical Research and the Program of the Institute: Mutagenesis and carcinogenesis are complex processes which may be related in their mechanism(s) of action. In the last few years, as more knowledge has been accumulated about the structure and metabolic fate of chemicals with respect to their biological activity, a general qualitative correlation between mutagenicity and carcinogenicity has been suggested. Additionally, mutagenicity test systems are also being investigated as potential prescreens to detect potential carcinogenic activity.

EMIC is correlating the availability of carcinogenesis information with the 1,500 compounds presently in its data base. This correlation along with EMIC's indexed literature collection makes it possible for the program of the National Cancer Institute to respond to its own and other queries for experimental data. This activity will assist the NCI and its contractors in carrying out the responsibilities stated in the National Cancer Act of 1971 which is to "collect, analyze, and disseminate all data useful in the prevention, diagnosis, and treatment of cancer including the establishment of an international cancer research data bank to collect, catalogue, store, and disseminate insofar as feasible the results of cancer research undertaken in any country for the use of any person involved in cancer research in any country."

Proposed Course: As the result of increasing interest in the correlation between mutagenesis and carcinogenesis, it is highly desirable that such a data base concerned with biological response to exogenous chemicals be maintained on a continuing basis.

The scientific literature shall be continuously monitored and all papers reporting the mutagenic effects of compounds in the human environment will be collected and added to the data base. Efforts will be continued to find more expedient methods of making this data file more readily available to interested individuals. Publication of selected data in tabular form will be continued. Reviews and special literature compilations will be done on areas of concern. Inputting of literature published before 1969 with respect to agents tested will be done to the extent that time permits.

Date Contract Initiated: June 30, 1972

Current Annual Level: $69,250

ASH STEVENS INC. (NO1-CP-23293)

Title: Synthesis of Purine and Pyrimidine Nucleotides

Contractor's Project Director: Dr. Calvin L. Stevens

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: The broad aim of this contract is to design and make available a new type of pharmacological agent or agent which will be useful in investigating the manner in which chemical and viral carcinogens affect the metabolism of newly-synthesized and messenger RNA in the cell nucleus, as well as for the investigation of a variety of other problems in chemical and viral

carcinogenesis. The specific aim of the contract is to synthesize suitably modified nucleotides which will irreversibly inactivate the nuclear enzyme which destroys newly-synthesized and messenger RNA in the nucleus itself; the agent must be selective and biologically active. An additional aim is that compounds which have been synthesized under this contract be assayed for possible inhibition of the RNA-dependent-DNA polymerase (reverse transcriptase) of oncogenic viruses and leukemic cells. This aspect of the project has been implemented and has yielded important results.

Major Findings: Chemical synthesis work in the past year has emphasized two major areas directed at a broad spectrum of active-site-directed irreversible inhibitors: 1) inhibitors derived from thymidylate oligomers bearing a neutral haloacetamido in the 3'-position and 2) inhibitors derived from thymidylate and 2'-O-alkyluridylate ribonucleotides bearing haloacetamidophenyl phosphoryl groups variously positioned at the 2'- or 3'- positions at the righthand end of the molecule and/or at the 5'-position at the lefthand end of the molecule. A third major phase, just getting underway, involves the enzymatic preparation of 2'-O-methylribonucleotide polymers, both homo and hetero, directed at the acquisition of long chain nucleotides as carriers of the reactive groups either terminally or along the polymer chain. In 1973, some 45 compounds were prepared in which the chain length was extended to hexamers (phase 1) and tetramers (phase 2). Inhibition data against the enzymes of the various above-cited categories are pending for many of the new compounds, but activity (to 10^{-6}M in some cases) and specifically (one or two orders of magnitude) have been demonstrated. Marked further progress in both respects is expected as the work is extended to higher oligomers and long chain strands of natural substrates for the target enzymes.

Significance to Biomedical Research and the Program of the Institute: Within the past few years, there has accumulated an important body of data which show that, in many different vertebrate cell types, most of the RNA that is made in the cell nucleus is destroyed in the nucleus itself very shortly after synthesis. Published data indicate that as much as 90% of the newly-synthesized RNA is destroyed in the nucleus itself and that its half-life may be only a few minutes. This entire field is still virtually untouched with respect to the problem of carcinogenesis. It is well established that chemical carcinogens have immediate and profound effects on the metabolism of RNA in the cell nucleus. The development of a selective drug probe, which would enable the investigator to stop the destruction of messenger RNA in the cell nucleus (without interfering with its synthesis), would open up a wide variety of experimental work in the field of chemical or viral carcinogenesis. Moreover, the synthesis and testing of potential inhibitors of reverse transcriptase, as well as other important cellular enzymes, a field in which this contract has already yielded successful results, are areas of high priority and relevance in other aspects of the NCI total program.

Proposed Course: To continue to develop new synthetic polynucleotide analogs by utilizing enzymatic synthesis to obtain longer chain length oligomers as carriers of the reactive (irreversible-inhibiting) groups either at the terminal or internucleotide positions. In addition, direct chemical introduction of reactive groups into suitable high molecular-weight synthetic polymers, which are the natural substrates of a given target enzyme, will be

studied. The compounds will be evaluated against the nuclear ribonuclease and other important target enzymes which may play a role in carcinogenesis.

Date Contract Initiated: June 21, 1972

Current Annual Level: $147,629

FRANKLIN INSTITUTE RESEARCH LABORATORIES (NO1-CP-33309)

Title: Preparation of Carcinogenesis Abstracts Vol. XI

Contractor's Project Director: Dr. Bruce H. Kleinstein

Project Officer (NCI): Dr. Sidney Siegel

Objectives: To provide an up-to-date abstract service for carcinogenesis researchers throughout the world in a format designed to provide under one cover scientific work in the various disciplines which relate to cancer etiology.

Major Findings: This contractor has performed well and delivered the required product on time.

Significance to Biomedical Research and the Program of the Institute: The multidiscipline approach used in the study of carcinogenesis needs an information exchange medium which gathers the relevant articles under one cover and once done allows for intellectual cross fertilization among the disciplines to produce a more rapid evaluation and use of information in the study of carcinogenesis.

Proposed Course: To continue the publication for an indefinite period of time and to include all relevant material in each issue instead of a set number of abstracts and/or citations.

Date Contract Initiated: February 1, 1973

Current Annual Level: $99,691

FRANKLIN INSTITUTE RESEARCH LABORATORIES (NO1-CP-43268)

Title: Information Resource

Contractor's Project Director: Dr. Paul N. Craig

Project Officer (NCI): Dr. Sidney Siegel

Objectives: This project is a resource providing the capability of rapid responses to information problems to the Carcinogenesis Program.

Major Findings: By the use of a basic ordering agreement, it has been possible to structure information tasks to fit individual problems. Each task is

defined by the requestor, the project officer and the contractor's project director. Projects which have been initiated to date include: simple screenings of the scientific literature for relevant data and information, recurring information packages on selected narrow areas of research, and the gathering, collating and evaluating of data and information on areas of research which have short and long-range implications on decision making for the scientist and administrator.

Significance to Biomedical Research and the Program of the Institute: All aspects of scientific research have information needs ranging from simple searching of the current literature to the preparation of critical reviews which are necessary for the optimal development of a research program. There has been a phenomenal increase in the volume of published literature in the past several years including a proliferation of journals. Carcinogenesis research includes a number of areas of science which compounds the information problem. Much time and effort can be expanded by an individual investigator in pursuing information needs, some of which can be readily handled and others for which the resources are not available but are necessary for the proper development of his research.

This project provides the resources for satisfying information needs of investigators and administrators in the Carcinogenesis Program in a timely fashion.

Proposed Course: The contractor will continue to respond to the information needs of the program.

Date Contract Initiated: October 25, 1973

Current Annual Level: $150,000

IIT RESEARCH INSTITUTE (NO1-CP-33296)

Title: Preparation and Characterization of Particulate Materials for Studies in Respiratory Carcinogenesis

Contractor's Project Director: Mr. Reg Davies

Project Officer(s) (NCI): Dr. John A. Cooper II
Dr. David Kaufman

Objectives: (1) to prepare and characterize any powder fractions needed for lung retention studies; (2) to prepare and characterize powder fractions coated with any polynuclear hydrocarbon carcinogen of interest to the National Cancer Institute, (3) to prepare and characterize dispersions of coated and uncoated particles in any vehicle or solvent of interest to the National Cancer Institute.

Major Findings: Size fractions of carbon and the oxides of iron, aluminum, nickel and cobalt have been prepared and characterized. These have been successfully coated with benzo(a)pyrene (BP) and maintained in a stable form for periods up to three months. Dispersions of BP in saline and gelatin have

been prepared and characterized.

These preparations, numbering close to 2,000, have been distributed to the institutions currently performing studies under the NCI respiratory carcinogenesis program.

Significance to Biomedical Research and the Program of the Institute: The significance of the contract is that the various NCI contractors have a constant and dependable source of materials for their biological studies. This ensures that each contractor performing related experiments has standard uniform materials for intratracheal instillation experiments.

Proposed Course: To continue this service to NCI for as long as it is required.

Date Contract Initiated: June 10, 1971

Current Annual Level: $75,000

IIT RESEARCH INSTITUTE (NO1-CP-33383)

Title: Stability of Benzo(a)pyrene on Particulates Utilized in Respiratory Tract Studies

Contractor's Project Director: Dr. Morton J. Klein

Project Officer (NCI): Dr. John A. Cooper II

Objectives: The Carcinogenesis Program conducts a number of studies directed toward the investigation of carcinogenicity of polyaromatic hydrocarbons (PAH). In a number of these studies the PAH is administered by the intratracheal route in the presence of particulate materials. Benzo(a)pyrene (BP) is the PAH most commonly used and the particulates are usually metal oxides of high purity having well characterized size, shape, and surface area. The particulates (either coated with BP or uncoated) are supplied to program investigators as the product of an ongoing contract activity.

The fact that BP has been reported to be altered by the effects of air or ultraviolet radiation and catalytically active metal oxide surfaces has caused concern as to the nature and composition of the final preparations which are being administered. This project will attempt to define the extent of BP degradation or modification that occurs between the time it is placed in contact with metal oxide particles and the time of animal administration. The experiments will be designed to identify and quantitate both stable products and those species which are stabilized on the metal oxide surfaces. The results of these studies would be used to define the optimal condition for production and storage of the particulates.

Major Findings: The most important finding was that, contrary to common belief among investigators in this field, BP alone or coated on hematite, is exceedingly stable. No measurable decomposition, by any analytical method, was observed, even after 110 hours at room temperature exposed to a bank of four 15 watt Sylvania Germicidal Lamps. Thus, although there appears to be no

problem in the storage and handling of BP samples, an important new consideration has been discovered. It has been heretofore generally accepted that the best method for disposing of spilled BP and for decontamination of equipment contaminated with BP was exposure to UV light. This practice must be abandoned immediately and new methods developed for decontamination. The BP does lose its fluorescence when it is exposed to UV, but this is apparently only a surface phenomena which is not analytically detectable.

A second discovery of importance is that the commercial BP being used varies in purity with the lot received, not only quantitatively, but also qualitatively as regards the nature of the impurities. A study of past data should be made to see if carcinogenic data can be correlated with the lot number of the material used.

Significance to Biomedical Research and the Program of the Institute: A number of studies utilizing polyaromatic hydrocarbons are being conducted by the Carcinogenesis program. The results of this study indicate that storage and handling do not present a stability problem. On the other hand, a serious problem of decontamination does exist, since the UV destruction technique is not valid.

In addition, the variability in the amount and nature of the impurities present in various BP lots may have considerable significance if any of these impurities are carcinogenic.

Proposed Course: In view of the initial findings, the immediate objective is to use analytical techniques developed to identify and quantify the impurities in BP. Experiments are also being designed to investigate the nature of the BP in immediate contact with particulate, since the bulk coating shows no changes. The various fluorescent colors of BP crystals do not appear to correlate with other forms of analysis. This anomoly is being examined microscopically.

Date Contract Initiated: June 1, 1973

Current Annual Level: $39,192

INSTITUTE OF LABORATORY ANIMAL RESOURCES (NAS) (NO1-CP-33338)

Title: Partial Support for the Institute of Laboratory Animal Resources

Contractor's Project Director: Dr. Robert Yager

Project Officer (NCI): Dr. Thomas Cameron

Objectives: To develop standards for the long-term holding of laboratory rodents (mice, rats, and hamsters) that could be used in all long-term studies of the Carcinogenesis Program. This document could become part of all RFP's involving long-term holding of rodents, and become a criteria by which unqualified proposals can be quickly and efficiently rejected and successful proposals can be held to a satisfactory level of performance. The standards will include

material from the proceedings of the Workshop on Criteria for Successful Chronic Rodent Studies, sponsored by the Carcinogenesis Program which was held April 4-5, 1973.

Major Findings: The ILAR has assembled a committee of experts familiar with the various factors (environmental, nutritional, disease, etc.) involved in long-term holding of rodents. The committee members are assembling data within their various field of expertise for incorporation into a standard.

Significance to Biomedical Research and the Program of the Institute: At the present time, no defined and documented protocol for the long-term holding of laboratory rodents exists. The development of such a protocol will define and document the criteria essential for the care, maintenance, and monitoring of the laboratory rodent used for long-term studies, such as the bioassay program, thereby providing reproducible and interpretable data.

Proposed Course: Production of an ILAR standard entitled "Standards for the Long-Term Holding of Laboratory Rodents." In the furtherance of the objectives of the animal resources activities of the Carcinogenesis Program, ILAR will continue its program for the collection and subsequent dissemination of well-substantiated information on genetically unique strains of laboratory animals including those inbred strains or outbred stocks which qualify as models for particular human diseases. As a corollary to the accumulation of information on genotypic entities, the staff of ILAR maintains an information center that responds to inquiries on a multitude of aspects such as laboratory animal care, breeding, nutrition, management and utilization. This information bank is readily available to the Carcinogenesis Program.

Date Contract Initiated: June 1, 1973

Current Annual Level: $20,000

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP-43273)

Title: Program for the Evaluation of Carcinogenic Risk of Chemicals to Man

Contractor's Project Directors: Dr. Lorenzo Tomatis
Dr. Claus Agthe

Project Officers (NCI): Dr. John A. Cooper II
Dr. Sidney Siegel

Objectives: The increasing number and quantity of chemicals introduced into our environment and their possible adverve effect, especially with regard to carcinogenicity, call for a coordinated effort to assess the magnitude of the resulting risk. Such an assessment makes it possible for preventive measures to be taken. The International Agency for Research on Cancer (IARC) with the support of the National Cancer Institute (NCI) has therefore established a program to evaluate the carcinogenic risk of specific chemicals to man. The objective of this program is to collect, evaluate and disseminate the existing information in this area.

Major Findings: Monographs are being prepared on chemicals for which there is significant human exposure and for which there is some published evidence of carcinogenicity in animals and/or man. They will be revised if and when new data and/or new principles for evaluation become available. So far four volumes have been published covering a total of 114 substances and two additional volumes are now in preparation.

In order to make the most effective use of the limited laboratory facilities available for testing, IARC in cooperation with NCI has initiated a world-wide survey of chemicals being tested for carcinogenicity. The information received from this survey is made available to all participating laboratories in the form of an information bulletin, in order to coordinate testing efforts. The first information bulletin (covering 176 compounds) was published in August and the second (covering 159 compounds) in December 1973.

Significance to Biomedical Research and the Program of the Institute: (1) The IARC represents a unique resource as a virtue of its easy communication with nations which sometimes could not otherwise freely exchange views. It is most likely that only such an international organization could establish either the registry of chemicals under test or the expert committees desirable for the production of the proposed monographs. (2) This activity will assist NCI in carrying out the responsibilities stated in the National Cancer Act of 1971 to "collect, analyze, and disseminate all data useful in the prevention, diagnosis, and treatment of cancer including the establishment of an international cancer research data bank to collect, catalogue, store, and disseminate insofar as feasible, the results of cancer research undertaken in any country for the use of any person involved in cancer research in any country."

Proposed Course: In an attempt to meet the specified objectives, data sheets in a condensed and uniform format are prepared on the individual compounds to be considered. The data sheets are utilized by the individual expert (or experts) designated to prepare a monograph on the chemical in question. These draft monographs are either discussed in small meetings or circulated by mail for comment during the course of their preparation. When the final draft is completed it is considered by a working group and any necessary revisions are made prior to publication.

Date Contract Initiated: June 30, 1972

Current Annual Level: $120,000

NCI FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc.)(CN-23294)

Project 9

Title: Preparation and Characterization of Carcinogens

Contractor's Project Manager: Dr. Walter Zielinski

Project Monitor (NCI): Dr. Marcia D. Litwack

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis.

NCI FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc.) (CN-23294)

Project 12

Title: Production of Inbred and Hybrid Laboratory Animal Strains

Contractor's Project Manager: Dr. Raymond Ediger

Project Monitor (NCI): Dr. Thomas Cameron

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis

NCI FREDERICK CANCER RESEARCH CENTER (Litton Bionetics, Inc.) (CN-23294)

Project 15

Title: Procedures for Safe Handling of Chemical Carcinogens

Contractor's Project Director: Mr. Everett Hanel (Acting Director)

Project Monitor (NCI): Dr. Larry Keefer

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis.

MASSACHUSETTS GENERAL HOSPITAL (NO1-CP-33297)

Title: Atlas on Comparative Morphology and Classification of Spontaneous Neoplasms in Dog, Cat and Man

Contractor's Project Director: Dr. Alan Schiller

Project Officer(s) (NCI): Dr. Sidney Siegel
Dr. Thomas Cameron

Objectives: To create a series of monographs on spontaneous and induced tumors encountered in the dog and cat, together with information on their histopathology, epidemiology, and etiology, with emphasis on comparative aspects of similar neoplasms of man.

Major Findings: During the third year of this contract these were the major achievements: (1) monographs on respiratory tract and thyroid neoplasms have been completed and submitted to reviewers for evaluation; (2) proposed monographs on pancreatic and hepatic tumors are currently in progress; (3) fine structure (electron microscopy) of a number of spontaneous dog and cat neoplasms is being studied.

Significance to Biomedical Research and the Program of the Institute: The projected use of these books would be to provide (1) a consolidated morphological and biological description and illustration of spontaneous neoplasms

in the dog and cat with an accompanying bibliography and standardized nomenclature, (2) a description of new spontaneous and experimentally produced canine and feline tumors heretofore unreported, and (3) comparative study with human neoplasms for use in selection of animal models for research.

Proposed Course: This is a cooperative project between human pathologists at the Massachusetts General Hospital and veterinary pathologists at the Angell Memorial Animal Hospital. Major emphasis will be on performing the histological examinations and obtaining data from animal case histories. Each monograph will deal with a specific organ system or organ depending upon the quantity of data available.

Date Contract Initiated: May 1, 1971

Current Annual Level: $55,000

MEDIZINISCHE HOCHSCHULE HANNOVER (NO1-CP-12148)

Title: Development of a Lung Tumor Model with Large Wild European Hamsters (*Cricetus Cricetus L.*)

Contractor's Project Director: Dr. Ulrich Mohr

Project Officers (NCI: Dr. Thomas Cameron
Dr. Norbert Page

Objectives: This contract was initiated to explore the possible usefulness of the European hamster (*Cricetus Cricetus L.*) as an animal model for the induction of respiratory tract tumors morphologically similar to tumors found in man. The specific aspects are to (1) capture the wild hamsters and establish a breeding colony, (2) determine the incidence of spontaneous tumors, especially bronchogenic carcinomas, and (3) test for sensitivity to induction of lung cancer by known carcinogens.

Major Findings: Studies conducted during this past year indicate that the wild European hamster is sensitive to the induction of bronchogenic carcinoma. Exposure to diethylnitrosamine and dibutylnitrosamine resulted in a high incidence of bronchogenic carcinoma as well as other tumors in the respiratory system. While these compounds also induce similar response in the Syrian golden hamster the relative incidence of bronchogenic carcinoma is greater in the wild European hamster. This was found to be especially true for dibutylnitrosamine. Exposure to nitrosomorpholine resulted in a high incidence of tumors in the nasal cavity, oral cavity and lungs. Nitrospiperidine induced high frequency of squamous cell carcinomas originating from the gingiva propria.

Hamsters obtained from a rural area have been adapted to the laboratory and have been bred. An F_4 generation is now being raised for further development of a breeding colony as well as an inbred colony. Considerable success has been accomplished in the domestication and breeding of this species.

Eight known carcinogens are being tested in the wild caught animals. LD50's and tolerated doses have been determined and are being administered in chronic

studies. Several papers have been published describing the results.

Significance to Biomedical Research and the Program of the Institute: Regardless of the causative factors of the bronchogenic carcinoma, having available laboratory animals with this characteristic would be invaluable to cancer research. Other characteristics which would make this animal superior to the Syrian golden hamster are its larger size and its longer lifespan. At present there is no satisfactory animal model for study of the natural pathogenesis bronchogenic squamous cell carcinoma.

While this type of tumor can be induced in the Syrian golden hamster via intratracheal instillation of chemical carcinogens, they do not spontaneously develop these tumors. Consequently, it is necessary to have reservations in extrapolating data obtained with the Syrian hamster to man. In addition, the use of an animal that is subject to the spontaneous occurrence of squamous cell carcinoma might be more suitable to studies of lung cancer using a natural exposure method, i.e., inhalation of aerosols.

The program is of direct and intimate relevance to the lung cancer program. If this species can be bred and maintained as a laboratory animal, it would be a valuable animal for the bioassay programs as well as to study mechanisms and pathogenesis of spontaneous lung cancer.

Proposed Course: Initially it was necessary to capture and adapt this wild species to laboratory conditions and develop a breeding program. The natural occurrence and incidence of diseases, especially lung tumors, has to be determined as this animal has a relatively longer lifespan than the commonly-used laboratory rodents.

The chronic testing of known carcinogens by intratracheal instillation and inhalation will be pursued during the coming year.

Date Contract Initiated: June 22, 1971

Current Annual Level: $57,000

MOUNT SINAI SCHOOL OF MEDICINE (NO1-CP-43255)

Title: Identification of Human Populations at Risk Due to Exposure to N-nitroso Compounds

Contractor's Project Director: Dr. William J. Nicholson

Project Officer(s) (NCI): Dr. Larry Keefer
Dr. Thomas Fears

Objectives: 1) To seek human populations which have had significant exposure to N-nitroso compounds, or high exposure to nitrates or nitrites which may promote the production of N-nitroso compounds in the human body. 2) To seek information on the extent of exposure for each specific population identified. 3) To develop protocols for epidemiologic investigations to assess increased

tumor risk in appropriate groups that have been identified. 4) To determine the feasibility of conducting studies based on the above protocols. Where possible, a sample of the defined population shall be studied or traced to determine the suitability of a complete study.

Major Findings: A variety of specific group exposures to N-nitroso compounds have been identified to date. These include the employees of chemical manufacturers, rubber industry employees, and students exposed during laboratory classwork. In each case the extent of the exposure, the number of individuals involved and the suitability of the group for future epidemiological study is being investigated. A small group of individuals, heavily exposed to dimethylnitrosamine 20 years ago, during the development of a dimethylhydrazine rocket fuel facility, has also been identified and the majority of these individuals traced.

Significance to Biomedical Research and the Program of the Institute: N-nitroso compounds have been shown to be acutely toxic and carcinogenic in animal studies, though little is known of their effect in humans. The disclosure that N-nitroso compounds can be found in food products and also synthesized *in vivo*, however, lends urgency to a determination of their effects in humans. The identification of industrial populations exposed to these compounds at levels much higher than those encountered in environmental circumstances offers the opportunity of demonstrating either the existence of or absence of human health effects from such exposures.

Proposed Course: The identification of exposed populations is to continue through May. At the end of this period, the development of protocols for epidemiologic investigations and the assessment of the feasibility of more complete studies will take place.

Date Contract Initiated: October 15, 1973

Current Annual Level: $38,960

STANFORD RESEARCH INSTITUTE (NO1-CP-33285)

Title: A Research Program to Acquire and Analyze Information on Chemicals That Impact on Man and His Environment

Contractor's Project Directors: Dr. Arthur A. McGee
Dr. Kirtland E. McCaleb

Project Officer (NCI): Dr. John A. Cooper II

Objectives: To develop a system to collect, analyze, and systematize information on the chemical description, production, distribution, and human exposure of chemicals with which the U.S. population comes in contact.

Major Findings: The present data base contains information on 3,500 chemicals in the following categories: intentional food additives, air pollutants, pesticide residues in food, water pollutants, proprietary drugs, soaps and detergents, prescription drugs, trade sales paints, and cosmetics. These

categories are subdivided into 900 product types representing 18,000 chemical-product combinations.

The data are in computer-readable form and contain the following information:

Product	Each Ingredient Chemical
Product name	CAS number and name
Quantity available for exposure	Strength (percent) in each product
Exposure routes -- oral, dermal, respiratory, and parenteral	Degree of uncertainty associated with quantitative data
Exposure factor by route	References to data sources

A computerized chemical classification scheme has been developed which contains 220 nodes or end points. A node assignment has been made for each of the chemicals for which exposure estimates have been made. As additional biologica data become available it may ultimately be possible to make reasonable estimates of a molecule's carcinogenic potential based on structure.

Also in computer-readable form are data on 25,000 chemicals to which the U.S. population is most likely to be exposed. These chemicals were drawn from eighteen recognized sources of information on such products as cosmetics, food additives, medicinals, etc. For each of the 25,000 chemicals, the following information is available: chemical identifiers (CAS number, structural representation, chemical name and synonyms), the node of correspondence in the chemical structure classification scheme, activity parameter estimates and uncertainty factors for each of the four routes of exposure.

Under this project, information on chemical production, distribution, use, and exposure is being provided to the International Agency for Research on Cancer for inclusion in the "IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man."

Developmental tasks under consideration include (1) analyzing trends in chemical production, exposure, and use; (2) further work in representing structure-activity relationships; (3) refining exposure factors; (4) including data on high risk subpopulations; (5) enlarging the data base to include other chemical physical, and biological information; (6) expanding the data base to include additional exposure categories and classes of chemicals; (7) reprogramming the data processing system to handle a larger data base and to respond to a greater range of inquiries; and (8) expand the system to serve other groups within NCI and other agencies, with regulatory and standard-setting responsibilities, concerned with health hazards arising from exposure to chemicals.

Significance to Biomedical Research and the Program of the Institute: The system being developed through this study provides information on many chemicals of interest both to the Carcinogenesis Program and to other programs concerned with the impact of chemicals on man.

Proposed Course: The project will continue as outlined above. It will continue to provide data useful for the selection of compounds to be introduced into the Carcinogenesis bioassay program. The information needs of the

following activities will be determined and incorporated into the system where feasible: (1) activities in the overall Carcinogenesis Program and in other organizational elements within NCI, and (2) activities within other Government programs concerned with the health hazards arising from exposure to chemicals.

Date Contract Initiated: October 30, 1970

Current Annual Level: $455,408

STARKS ASSOCIATES, INC. (NO1-CP-23203)
(Supplement to Division of Cancer Treatment for Animal Production)

Title: Procurement, Purification, Characterization, and Distribution of Standard Reference Compounds for Carcinogenesis Bioassay

Contractor's Project Director: Dr. Fred Starks

Project Officer (NCI): Dr. Elizabeth K. Weisburger

Objectives: The contractor is to acquire stated quantities of specific compounds in a high state of purity and repackage in smaller quantities. The compounds will be stored and distributed to contractors and others working in cancer research who do not have facilities for preparation or purification of chemical carcinogens required to meet program needs.

Major Findings: Prior to the publication of the Occupational Safety and Health Act temporary standard for handling carcinogens, safety procedures and physical facilities were installed. A rather limited modification of the facilities resulted in full compliance with the OSHA regulations.

Seventy-seven standard reference compounds have been transmitted to the National Cancer Institute. These standards were subdivided into multiple units ranging in size from 5 mg. to 100 g. Packaging containers and shipping methods were devised to retain the chemical integrity of the compounds. All materials were packaged under argon and shipped in appropriate containers sealed with teflon lined covers.

Fifty-one of the compounds supplied had a minimum purity of 99%. Eight of the materials were acceptable standards at a purity of 97-98%. Chemical constitution and purity of each product were defined by a combination of elemental analyses, assay methods, trace element analyses, thin-layer chromatography, gas liquid chromatography, and spectrophotometric characteristics. Information was supplied for each material describing the analyses, synthetic approach, and purification methods. Eighteen of the compounds, obtained from commercial sources, were transmitted directly to contractors of the National Cancer Institute without determination of purity.

Approximately 15% of the standards were synthesized. Commercially available compounds were refined to meet the 99% minimum purity standard.

Significance to Biomedical Research and the Program of the Institute: In order to establish a quantitative basis for comparison of carcinogenic effects observed in various laboratories, it is important that standard reference materials be used. The availability of such materials will facilitate several phases of research on chemical carcinogenesis. Programs especially effected would be those aimed at identifying carcinogenic hazards for man, biochemical studies on the effects of chemical carcinogens, and prevention or modification of the effects of carcinogens. High purified and well-characterized chemical compounds to be acquired include known carcinogens, non-carcinogenic structural analogs, and selected metabolic products.

Proposed Course: The chemicals provided by the contractor will be used by the Bioassay Operations Segment and other programs wherever needed.

Date Contract Initiated: June 21, 1972

Current Annual Level: $78,000

TRACOR JITCO, INC. (NO1-CP-33402)

Title: Production of 1972-1973 Volume of "Survey of Compounds Which Have Been Tested for Carcinogenic Activity" and Production of Machine Readable and Searchable Indexes of the PHS-149

Contractor's Project Director: Mr. Anthony Lee

Project Officer (NCI): Dr. Sidney Siegel

Objectives: To compile and publish data from the 1972-1973 scientific literature on the carcinogenic activity of chemicals in experimental animals. The resultant publication will be the 1972-1973 Volume of the PHS Publications Series No. 149, entitled Survey of Compounds Which Have Been Tested for Carcinogenic Activity.

Major Findings: This activity represents a continuation of the work carried out under NIH Contract 69-2086 and 71-2266, in which volumes covering the 1961-1967, 1968-1969, and 1970-1971 scientific literature were prepared.

The contractor has searched the scientific literature, selected appropriate documents, extracted and placed in a formal matrix specific data from these documents, and has indexed selected items from the extracted data. Indexes were generated for (1) literature reference, (2) route of administration, (3) site of application, (4) animal species/strain, (5) tumor site, (6) vehicle used, (7) chemical names of compound administered, (8) Chemical Abstracts Registry (CAS) number of the compound administered, (9) molecular formula of compound administered, and (10) Wiswesser line notation of the compound administered.

Cumulative indexes of the first six PHS-149 Volumes are also being converted into a computer readable data base, structured to allow maximum information

retrieval using Boolean algebra logic with the chemical and biological search terms.

Significance to Biomedical Research and the Program of the Institute: The 1972-1973 Volume of PHS Publication No. 149, will bring the series of volumes up-to-date and provide a tool useful in searching the chemical carcinogenesis literature. The conversion of the indexes to a machine searchable data base will enhance the utility of this information.

Proposed Course: This contract will be terminating as soon as the 1972-1973 volume is delivered.

Date Contract Initiated: June 30, 1973

Current Annual Level: $114,366

WOLF RESEARCH AND DEVELOPMENT CORPORATION (NOI-CP-43257)

Title: Carcinogenesis Bioassay Data System (CBDS) Operational Support

Contractor's Project Director: Mr. Dalton Tidwell

Project Officer (NCI): Mr. T. D. C. Kuch

Contract narrative is reported under the Bioassay Operations Segment.

SUMMARY REPORT

LUNG CANCER SEGMENT

July 1, 1973 through June 30, 1974

The Lung Cancer Segment is devoted to studies of the pathogenesis of lung cancer and its prevention. The ultimate goal of the program is the prevention of development of lung cancer in man. A variety of experimental techniques are used in many investigations involving long-term animal studies, as well as short-term morphological and biochemical studies, all of which are coordinated to establish a correlation between animal models and human lung cancer and to develop a targeted approach to the inhibition or prevention of this most prevalent form of fatal cancer in man. The need for such a program to lessen the incidence of this widespread, lethal disease scarcely requires further comment. During the past year major new investigations in the area of *in vitro* carcinogenesis in respiratory epithelium and carcinogen metabolism in respiratory epithelium have been developed. Significant accomplishments of the entire program during the past year are described below.

In the "Oak Ridge Respiratory Carcinogenesis Program" (AEC - Oak Ridge National Laboratory), the pathogenesis of lung cancer is being investigated in a number of long-term animal studies that are of direct relevance to the human disease. The potential co-carcinogenicity of several irritant gases, that are common combustion products (such as nitrogen dioxide) or are prevalent in the gas phase of tobacco smoke (such as formaldehyde), is being tested in rat and hamster lung tumor models. The toxic response of the respiratory tract epithelium to these irritants is also under investigation. Studies have been performed to investigate the effects of both high and low vitamin A intake on the induction and growth of lung cancer. It has been confirmed that administration of high doses of vitamin A to rats partially protects the respiratory tract epithelium from the tumorigenic effects of a polynuclear hydrocarbon such as methylcholanthrene. A new system for grafting tracheas into recipient animals has been developed, and it has been found possible to induce carcinomas in the grafts by suitable application of carcinogen.

In "Studies on Pulmonary Carcinogenesis," (New York University School of Medicine, Department of Environmental Medicine), the major emphasis is on the identification of hazardous materials which may pose a serious threat to man as respiratory carcinogens. Great effort has been spent in the design and establishment of an inhalation chamber facility which can accommodate over 1,000 animals. Systems for the generation of aerosols, vapors, and industrial dusts have been developed for testing the carcinogenicity of many substances by the inhalation route. In last year's report, it was noted that among the important substances which have been found to be carcinogenic or co-carcinogenic for the respiratory tract are calcium chromate dusts, sulfur dioxide, dusts formed from polyurethane

foam (workers in the building trades industry may be exposed to this type of dust), and bis-chloromethyl ether, a common industrial chemical. New substances for which testing has begun this year include alkylating agents widely used in industry, as well as wood dusts that have been suspected as an occupational hazard; further studies have also begun on chrome process materials actually used in commercial chromium refining. Results obtained from this contract have already been used by the Department of Labor to help set standards of exposure for industrial workers to occupational carcinogens.

In "Factors Influencing Experimental Respiratory Carcinogenesis by Alpha Radiation and Chemical Carcinogens," (Harvard University School of Public Health), the potential synergism between radiation and chemical carcinogens in causing lung cancer is being studied. Such synergism has been implicated in the high incidence of lung cancer in uranium miners. The animal studies in the present contract will take several years to complete. Recent evidence just obtained in this program suggests that there is indeed a strong synergism between polonium-210 (which is present in cigarettes) and benzo[a]pyrene, in terms of their ability to induce lung cancer in the hamster.

In"Susceptibility States and Modulating Factors in Respiratory Carcinogenesis," (IIT Research Institute), genetic, as well as enviromental, factors are being assessed for their influence on development of lung cancer in experimental animals. The carcinogenicity of benzo[a]pyrene for the respiratory tract of different strains of inbred hamsters is being measured; it is hoped to identify strains that are highly susceptible or highly resistant to development of lung cancer. This study has human relevance, for if biochemical and cellular differences can be identified in these animal experiments, significant leads may be found for eventual assessment of the risk of individual human beings to exposure to respiratory carcinogens. Another important factor which may modify host response to respiratory carcinogens is viral or bacterial infection of the respiratory epithelium; this is being evaluated in studies in which APR/8 influenza virus is administered together with benzo[a]pyrene. The ability of 13-cis-retinoic acid, a synthetic analog of vitamin A, to prevent the development of lung cancer in animals that have been previously treated with the carcinogen, benzo[a]pyrene, is being assessed in a major long-term study. Since the practical usefulness of natural vitamin A in preventing the development of lung cancer is limited by its intrinsic toxicity, it is hoped to develop and test new synthetic analogs of vitamin A for their ability to prevent lung cancer. The immediate goal is to develop a new, synthetic form of vitamin A that will be more potent and less toxic than natural vitamin A. The initiation of many of the above long-term animal studies was reported last year; within the coming year, final test results should be available in several projects. This contract is also using a new tool, the scanning electron microscope (SEM) to follow changes that occur in the respiratory epithelium during carcingoenesis; this instrument may have ultimate potential for improvement of diagnostic studies in man. The results of several of these SEM studies have just been published.

The results of many of the above animal studies are being utilized in several human studies in the Lung Cancer Segment. In the contract, "Morphogenesis of Human Lung Cancer," (St. Mary's Hospital, Grand Junction, Colorado), an intensive effort is being made to improve the technique of sputum cytology and to use it effectively for earlier diagnosis of lung cancer in man. A large group of uranium miners is being followed with sputum cytology, in order to achieve the earliest possible diagnosis of lung cancer in this population of workers who are at such high risk for development of lung cancer. An atlas of human sputum cytology is being compiled that will be of help in diagnostic centers throughout the country. In a collaborative study with workers at the Oak Ridge National Laboratory, a good correlation has been found between animals and man, in terms of the cytologic stages that occur during the development of bronchogenic squamous cell cancer. These studies indicate that findings made in the animal model should be of direct relevance to study of the human disease.

Since a significantly large number of deaths from lung cancer in man are caused by oat cell carcinoma (which is a particularly invasive and metastatic type of tumor), a contract, "Studies on Oat Cell Cancer of the Lung," (Stanford University School of Medicine) to investigate the morphological and biochemical properties of this type of cancer has been developed. A leading hypothesis is that oat cell carcinomas arise from a specific type of cell (K-type cells) in the respiratory tract. Studies in this contract are aimed at identifying the unique morphological and biochemical properties of the K-type of cells, as well as the factors of mechanisms involved in the neoplastic transformation of these cells. During the past year, several cell lines from human oat cell tumors have been established in tissue culture. In addition, electron microscopic examination of several lung cancers induced in hamsters with benzo[a]-pyrene has demonstrated secretory granules of the type found in human oat cell cancers. This is an important finding, because it suggests that it may be possible to develop an adequate animal model for this type of tumor.

Another project to study human tissue has just been started at the University of Maryland ("Studies on Normal, Premalignant, and Malignant Tracheobronchial Epithelium of Humans"). Human respiratory epithelium is being characterized by electron microscopic and cytochemical techniques,and is also being placed in organ culture in order to study its response to carcinogens. It has already been established that explants of human bronchi can be maintained for as long as 8 weeks in organ culture with good preservation of ultrastructural morphology. In addition, it has been found that in such explants benzo[a]pyrene binds tightly to the nuclear area of several different types of epithelial cells. This entire project will give valuable correlations between animal studies and the problem of lung cancer in man.

In order to further study the progression of preneoplastic lesions that will be induced in the previous project, a new program has just been started during the last part of this fiscal year at Litton Bionetics ("Studies of Carcinogenesis in Human Bronchial Tissue"). Human bronchial epithelial tissues bearing preneoplastic lesions will be transplanted and

maintained in vivo as xenografts in either the athymic nude mouse or the immunosuppressed hamster, in order to determine whether such lesions have the potential to progress toward invasive malignancy.

In order to effectively pursue many of the above studies on lung tissue from both man and animals, improved cytological methods, such as radioautography, are required. In the contract, "Autoradiographic Study of the Cellular Response of the Respiratory Tract during Chemical Carcinogenesis," (VA Hospital - Tampa, Florida), the technique of light microscopic radioautography of the respiratory tract has been improved, so that the actual amount of radioactivity in cellular and subcellular compartments of respiratory epithelium can be quantitatively measured. During the past year, it has been found that intratracheal administration of benzo[a]-pyrene, as well as vitamin A deficiency, causes an increase in cellular proliferation in respiratory epithelium, as quantitated by thymidine labelling. In addition it has been found that vitamin A deficiency causes loss of ciliated cells and an increase in the number of basal cells in respiratory epithelium. High doses of vitamin A cause an increase in ciliated cells. These studies indicate that vitamin A exerts an important effect on the cell population and cellular kinetics of respiratory epithelium.

Two new contracts have been initiated in the past year to study respiratory carcinogenesis in vitro ("Studies of Carcinogenesis in Organ Culture of Trachea and Bronchi"). At the State University of New York at Stony Brook, this phenomenon is being studied in tracheal rings from rats, while at the University of Vermont tracheal plaques from hamster are being used. In both projects, the investigators have already been able to maintain respiratory tissue for prolonged periods in vitro and to obtain significant cytologic alterations in the epithelium after treatment with carcinogenic hydrocarbons. Some of these treated explants have been transplanted back to intact animals to see if they will develop into tumors. Both projects have had an excellent start, and should yield valuable information about factors that either promote or inhibit the development of lung cancer. There is good communication between the two projects, and the joint effort should be mutually helpful.

Since it is well established that many carcinogens (including those which affect the lung) must be metabolically activated before they can exert their carcinogenic effects, a major new effort has been developed in this area within the past year. Such research has long-term potential to find effective means to block the conversion of inhaled or ingested carcinogens to metabolically active forms, and thus to inhibit the development of lung cancer. Three new contracts, all entitled "Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract" have been started. At the University of Minnesota, efforts are focused on the role of antioxidants, such as butylated hydroxyanisole (BHA), in modifying carcinogen metabolism. It has already been found that BHA can inhibit the binding of benzo[a]-pyrene to DNA, as mediated by lung or liver microsomal enzymes in vitro. In parallel long-term animal studies, the usefulness of BHA and related compounds in blocking development of lung cancer is being evaluated. At the

University of Stockholm, efforts are directed at defining the relationship between the microsomal enzyme system, aryl hydrocarbon hydroxylase, and lung cancer. This is a critical enzyme system for activation of carcinogenic hydrocarbons. Newer methods for assaying this enzymatic activity, which will enable more reliable micro assays on subcellular fractions from cells of the lung, have already been developed. It is also planned to perform such assays on human lung tissue, which will further elucidate the role of this enzyme system in man. Since the aryl hydrocarbon hydroxylase system is an extremely complex linkage of various molecular components, the investigators at the University of Texas (Southwestern Medical School at Dallas) are analyzing the role of the various molecular components in lung microsomes. Methods have been developed in the past year for preparation of microsomal fractions from both rat and hamster lung. The role of oxygen donors, cytochrome P-450 and cytochrome P-448, in these microsomal complexes is being characterized. These cytochromes represent the actual molecular interface at which the carcinogenic hydrocarbons may be converted to their active forms. It is apparent that a great deal of important basic biochemistry remains to be done on this problem, but the results are of great importance to our understanding of the initiation of lung cancer in man. Meetings are held frequently by the three groups of investigators working on this entire problem of polycyclic hydrocarbon metabolism, with a maximal sharing of information.

Since vitamin A has such a profound effect on cellular differentiation in respiratory epithelium, and since it has been shown to be capable of inhibiting carcinogenesis in this epithelium, the biochemical bases of these phenomena are being further investigated, with the long-term goal of achieving more effective use of vitamin A or vitamin A analogs in cancer prevention. In the contract, "Role of Vitamin A in the Control of Differentiation and Carcinogenesis in the Respiratory Tract," (Massachusetts Institute of Technology), the role of vitamin A in controlling the synthesis of specific glycoproteins in respiratory epithelial cells is being studied. Vitamin A has been found to be required for the synthesis of specific glycopeptides in these cells, both in the whole animal and in organ culture. These glycopeptides may be important determinants of membrane structures within the cell, and thus determine overall cellular morphology and physiology. The various parameters that affect the ability of vitamin A to block the development of lung cancer in the experimental animal are also being evaluated in this contract.

Since the intrinsic toxicity of natural vitamin A is a limiting factor in its usefulness in cancer prophylaxis, studies are underway to identify new synthetic analogs of vitamin A that will be more potent and less toxic than the parent substance. In the contract, "Organ Culture Assay of Vitamin A Analogs," (Southern Research Institute), such potential analogs are being evaluated, by both morphological and biochemical techniques. These assays have the important feature of providing rapid test data on unknown compounds. The morphological effects are being evaluated in organ cultures of chick skin and mouse prostate, while biochemical studies have employed tissue from both mice and hamsters

(liver and lung). In the organ culture assays, several modifications of the ring portion of the natural vitamin A molecule have been found to be more potent than the parent compound in inducing mucus metaplasia of chick skin. The vitamin A compounds have been evaluated as inhibitors of microsomal mixed function oxidases from liver and lung tissues of mice and hamsters. Some of the compounds are potent inhibitors of the enzymatic oxidation of benzo[a]pyrene and other carcinogenic polycyclic hydrocarbons to products which become tightly bound to reaction components. Alterations in the metabolism of polycyclic hydrocarbons by vitamin A compounds may be related to their anticarcinogenic action.

In summary, the Lung Cancer Segment is pursuing a coordinated program of studies at the human, animal, cellular, and molecular level, to investigate the factors which are involved in the cause and prevention of lung cancer. It is hoped that within this coordinated framework there will be the most rapid dissemination and utilization of new information obtained at the basic levels, and that we thus may hopefully be able to shorten the time before we achieve the desired goal of prevention of lung cancer in man.

CONTRACT NARRATIVES

LUNG CANCER SEGMENT

July 1, 1973 through June 30, 1974

AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory) (YO1-CP-40013)

Title: NCI-AEC Carcinogenesis Program

Contractor's Project Director: Dr. Francis T. Kenney

Project Officer (NCI): Dr. Allen Heim

Contract narrative is reported under the Office of the Associate Director for Carcinogenesis.

HARVARD UNIVERSITY SCHOOL OF PUBLIC HEALTH (NO1-CP-33273)

Title: Factors Influencing Experimental Respiratory Carcinogenesis by Alpha Radiation and Chemical Carcinogens

Contractor's Project Director: Dr. John B. Little

Project Officer (NCI): Dr. Norbert Page

Objectives: To examine some of the factors which influence the carcinogenic response to respiratory carcinogens, and to determine in particular whether a synergistic or potentiating effect exists between alpha radiation and chemical carcinogens in the production of lung cancer. Polonium-210 will be used as a local source of alpha radiation, benzo(a)pyrene (BP) as a local chemical carcinogen and diethylnitrosamine (DEN) as a systemic carcinogen.

Major Findings: Lung cancer has been induced in hamsters by the intratracheal instillation of either polonium-210 or BP adsorbed onto hematite particles. No tumors occurred in untreated or hematite-treated control animals. Distribution studies with polonium either in saline or on various concentrations of hematite particles indicate that the uniform distribution of alpha-emitting radionuclides as occurs following saline instillation is more carcinogenic than the non-uniform distribution or "hot-spots" seen with the particulate instillation. Localization studies utilizing freeze dried frozen sections and dry mount autoradiography have shown that intratracheally administered polonium-210 delivers its major dose to the epithelium of the distal airways, whereas BP localizes in the tracheal and bronchial epithelium. These are the regions where the malignant tumors induced by each agent arise. Preliminary results indicate that low doses of polonium-210 and BP act synergistically following sequential administration.

Significance to Biomedical Research and the Program of the Institute: The proposed studies are addressed to a very critical problem in the evaluation of human lung cancer hazards which may result from the combined effects of

radiation and chemical carcinogens. Synergistic relationships between alpha radiation and chemical carcinogens have been implicated in the high incidence of occupational lung cancer in man (uranium, hematite, and flurospar miners). Previous studies have shown that polonium-210, a component of tobacco smoke, accumulates in the bronchial wall, especially the segmental bifurcations of smokers. This is a region where a high percentage of the bronchial carcinomas are found. The possible role of polonium-210, acting as a cofactor along with other chemicals in tobacco smoke, needs clarification. Results from this project could have a direct bearing on the whole evaluation of lung cancer hazards in man, and on future developments in the whole Lung Cancer Program.

Proposed Course: Studies will be continued to assess synergistic effects between BP and polonium-210 administered simultaneously and sequentially. Techniques of administration will be sought which will deliver both carcinogens to the same site in the lungs. Future studies will continue to examine interactions between local carcinogens, and will also involve administration of DEN systemically following treatment with either BP or polonium-210 to determine possible synergism between local and systemic carcinogens.

Date Contract Initiated: May 30, 1971

Current Annual Level: $110,000 (Estimated)

IIT RESEARCH INSTITUTE (NO1-CP-92148)

Title: Role of Vehicles and Particulates in Respiratory Carcinogenesis Bioassays

Contractor's Project Director: Dr. Mary C. Henry

Project Director (NCI): Dr. David G. Kaufman

Objectives: To determine the effect of intratracheal instillation of particulates of varied chemical and physical properties and of various types of solvents on the induction of epidermoid carcinoma of the lung by chemical carcinogens in the hamster.

Major Findings: Chemically and physically defined particulates, produced by another contract at IIT Research Institute, (NIH-NCI-33296), were used in these studies. Long term carcinogenesis trials were conducted with preparations containing particles of different sizes and aggregates of carcinogen-dust with different physical properties. The effective dose of carcinogen was defined as the amount delivered through a syringe and/or present in hamster lung immediately after intubation.

The size of the dust particle, the size of the carcinogen-dust aggregates and physical association markedly influenced the onset and incidence of lung tumors. The probability of tumor-bearing animals at weeks of experiment was greater when benzo(a)pyrene (BP) was attached to small particles (0.5-5μm) than to larger sizes (5-30μm). A mixture of small and large ferric oxide particles with carcinogen produced a higher tumor incidence than the carcinogen larger particle preparation, but a lower incidence than BP attached to smaller particles.

Methods of preparation influenced the size of carcinogen-dust aggregates and development of epidermoid carcinomas. Larger aggregates were present after low-temperature precipitation of BP onto the dust than after grinding the two substances together in a mortar. The former preparation produced a faster onset and a greater probability of tumor-bearing animals. Simple mixing of the carrier dust and carcinogen in a vehicle did not produce a physical association and treated hamsters had a very low lung tumor yield. The presence of ferric oxide particles less than 0.5μm enhanced the induction of peripheral respiratory tract carcinomas. Without the carrier dust, only large doses of BP can initiate the neoplastic process.

The rate of BP elution from different size particles and aggregates in vivo was examined. Physical association of the carcinogen and dust slowed the lung clearance of BP but retention rates did not correlate with particle size. In this model system, the presence of the dust in the lung did not influence tumor induction by nitrosamines, whether given systemically or intratracheally. Lung tumors were induced by the intratracheal instillation of dimethylnitrosamine.

Studies utilizing scanning electron microscopy were directed toward the effects of BP-ferric oxide preparations on the morphology of respiratory epithelial surfaces in the trachea. Multiple instillation of ferric oxide produced a loss of ciliated cells and broad areas of abnormal, enlarged non-ciliated cells with roughened surfaces. Intubation of BP coated on ferric oxide caused similar changes and in addition, squamous metaplasia and small foci of markedly abnormal protuberant cells. Focal areas of squamous metaplasia in Vitamin A deficiency were documented by this technique. These focal areas were located on cartilage rings in the upper ventral portion of the trachea. The dorsal or membraneous portion was essentially unaffected. Upon reversal of the deficiency state, the entire epithelial surface sloughed and underlying basal cells differentiated into ciliated and non-ciliated cells.

Significance to Biomedical Research and the Program of the Institute: This project is one of several designed to develop techniques for studying the most common type of lung cancer found in man. It is based on previous observations that bronchogenic epidermoid carcinomas could be induced in hamsters by the intratracheal administration of benzo(a)pyrene with iron oxide dust. These studies have shown the importance of the physical characteristics of inhaled carcinogens in the development of epidermoid carcinomas.

Proposed Course: The carcinogenesis studies have been completed and a final report is being compiled.

Date Contract Initiated: June 27, 1969

Current Annual Level: $48,775

IIT RESEARCH INSTITUTE (NO1-CP-23292)

Title: Susceptibility States and Modulating Factors in Respiratory Carcinogenesis

Contractor's Project Director: Dr. Curtis Port

Project Officer (NCI): Dr. David Kaufman

Objectives: The objectives of this contract are to determine the following: (1) do states of increased or reduced susceptibility to the development of respiratory tract tumors exist in hamsters?, and (2) do conditions exist which, after the administration of a carcinogenic regimen, can modulate the ultimate tumor response in the respiratory tract of hamsters?

Major Findings: A study has been begun to determine if hyperplasia of the respiratory epithelium represents a state of increased susceptibility to carcinogenesis. A/PR/8 hamster-adapted influenza virus was administered intranasally and followed by an intratracheal instillation of gelatin-saline; this produced hyperplasia of tracheal, bronchial and bronchiolar epithelium. The optimal time interval between virus infection and gelatin-saline administration is between 12 and 18 hours as determined by ^{3}H-thymidine incorporation. Epithelial hyperplasia is first apparent in the trachea 3 days after virus infection and in the lung epithelium 4 days after virus infection and disappears in both areas by the 20th day. Studies to determine the rates of carcinogen clearance from the lungs of normal and virus infected animals have been carried out. The results indicate that a virus induced hyperplasia does not interfere with the clearance of BP from the respiratory tract. A life-time carcinogenesis study is currently in progress to determine whether there is an increased tumor incidence in the respiratory tract of hamsters that receive a series of intratracheal doses of (benzo(a)pyrene adsorbed to Fe_2O_3 during virus-induced hyperplasia as compared to normal hamsters receiving the carcinogen.

A technique has been developed that permits the intratracheal instillation of volumes as large as 1.5 ml into the respiratory tract of young adult hamsters. Aspects of the procedure which permit its success are (1) the use of an intratracheal speculum which seals at the larynx and thereby contains the instillation volume within the respiratory tract, and (2) the use of a rapidly acting anesthetic (halothane) which permits the animal to recover soon after the instillation is carried out. Pulmonary edema, exemplified by rales, occurs during the first 72 hr post instillation and is gone thereafter; histopathological changes seen at 4 weeks are slight. Volumes in excess of 1 ml are evently distributed to all lung lobes and damage to the larynx and trachea is minimal. A lifetime experiment is currently in progress to determine the toxicity and tumorigenesis associated with the administration of 30 mg BP-iron oxide in two instillations made 1 week apart by the large volume technique.

Since morphological changes are an integral part of the neoplastic process, the scanning electron microscope (SEM) has been used to define the process of squamous differentiation and its relationship to metaplasia and squamous carcinoma. The epithelial hyperplasia produced in the respiratory tract as a result of virus and gelatin-saline administration has been corroborated with the use of the SEM. Changes in surface morphology agree with ^{3}H-thymidine incorporation with respect to time course. Methods for SEM correlation with light microscopy or TEM are being investigated utilizing a variety of fixation techniques with plastic embedment for thick and thin sectioning. Also, methods of specimen preparation for exfoliated cells from the respiratory tract are

are being investigated. Current methodology is being reviewed and new techniques considered. Two additional studies have been initiated which utilize the SEM. The first, a BP-iron oxide tumor study, is a companion study to the ongoing large volume study discussed above. The cellular morphology in premalignant lesions will be compared with tumors. Bronchiolo-alveolar proliferation is the subject of the second study. The histogenesis of these lesions will be evaluated acutely following chemical injury to the lung. These lesions will be evaluated for potential investigation as a state of increased susceptibility to peripheral lung tumor induction.

A vitamin A analog study is in progress to determine the effectiveness of 13-cis-retinoic acid in reducing the number of carcinogen induced tumors of the respiratory tract. The animals have received two dose levels of BP, as a BP-iron oxide preparation, and two dose levels of vitamin A analog. Corresponding control groups have been included. To date, body weight and mortality are approximately the same for all experimental groups, illustrating that neither the analog nor the analog vehicle is toxic at the dosage given. No statement can be made as to the effect of the analog on tumor incidence at this time. A study to identify susceptible or resistant hamster inbred strains to chemical carcinogenesis has been initiated. Two potentially susceptible and one potentially resistant strain have been selected and will be compared with an outbred strain.

Significance to Biomedical Research and the Program of the Institute: The contract is one of an integrated program of contracts examining facets of the induction of respiratory tract tumors in animal models. Studies in this contract are intended to identify conditions which place experimental animals at reduced or increased risk to the development of respiratory tumors. These conditions may be exploitable for the development of preventative and/or therapeutic measures for respiratory cancer.

Proposed Course: The experiments described in the major findings section will continue, and a histopathologic evaluation of the type and location of the respiratory tumors produced will be made.

Date Contract Initiated: June 26, 1972

Current Annual Level: $350,000 (Estimated)

LITTON BIONETICS, INC. (NO1-CP-43274)

Title: Studies of Carcinogenesis in Human Bronchial Tissue

Contractor's Project Director: Dr. Clement C. Darrow II

Project Officer (NCI): Dr. Curtis Harris

Objectives: Using human lung tissues to study the development of preneoplastic (and possibly neoplastic) lesions induced by chemical carcinogens and to study the ability of selected agents to modify the effects of chemical carcinogens. Human bronchial epithelial tissues are to be exposed *in vitro* to chemical

carcinogens, as well as to anti-carcinogens. These tissues are then to be transplanted and maintained in vivo in the athymic nude mouse and immunosuppressed hamsters as zenografts.

Major Findings: As this is a new contract, no major findings can be reported at this time.

Significance to Biomedical Research and the Program of the Institute: The establishment of an animal host for human bronchial tissue xenografts which have been treated with carcinogens and anticarcinogens in vitro will provide a model more predictive of the effects of anti-carcinogens on neoplasia when used in man than trying to extrapolate from an animal tumor or tissue culture model.

Proposed Course: Proceed to implant xenografts of human bronchial tissue in the athymic nude mouse and immunosuppressed hamster to develop the long-term (up to one year) model desired. Given the model, test the effects of various carcinogens and anti-carcinogens with the system.

Date Contract Initiated: February 1, 1974

Current Annual Level: $106,845.

MARYLAND, UNIVERSITY OF (NO1-CP-43237)

Title: Studies on Normal, Premalignant and Malignant Tracheobroncial Epithelium of Humans

Contractor's Project Director: Dr. Benjamin F. Trump

Project Officer (NCI: Dr. Curtis C. Harris

Objectives: The objectives of this contract are twofold: (1) To characterize the normal morphology of the human tracheo bronchial epithelium in normal, premalignant and malignant states by light and electron microscopy and to perform selected cytochemical techniques, especially those related to the functions of the Golgi apparatus and cell surface in an attempt to characterize fully differentiated epithelium and to compare it with anamolies seen in premalignant and malignant states; and (2) To maintain in organ culture, human tracheobronchial epithelium removed surgically from patients with or without bronchial carcinoma or removed at immediate autopsy, and to study the epithelial morphology as a function of time in organ-culture.

Major Findings: The major finding at time of writing is that it is possible to maintain human bronchi for up to 8 weeks in organ culture with preservation of good ultrastructural morphology. Cultured epithelia are similar to epithelia fixed before culture and normal appearing columnar epithelium, showing ciliated and mucous cells, areas of basal cell hyperplasia, and areas of squamous metaplasia are seen. Studies to date also indicate that it is possible to store bronchi for at least 48 hours at 0-4°C prior to successful culture. The preservation of ultrastructural morphology correlates with

demonstrated active incorporation of tritiated leucine, uridine and thymidine into cellular macromolecules. At the cut edges undifferentiated low cuboidal cells extend around the explant and more of these cells show thymidine labeling when compared with mucous and ciliated areas.

Studies employing tritiated benzo(a)pyrene (BP) indicate tight binding of tritiated-BP in bronchial epithelial cells as measured by quantitative light microscopic autoradiography. Tritiated-BP was bound to basal, mucous, ciliated, and squamous metaplastic cells with a five-fold increase of binding as compared with binding in bronchial epithelial cells maintained at 0°C. About 50% of the grains overlay nuclei.

Significance to Biomedical Research in the Program of the Institute: The principal significance of these studies is that they will provide characterization at the ultrastructural level of normal and various premalignant and malignant lesions of the human bronchus. Moreover, the successful organ culture of human bronchi opens up new horizons in the study of carcinogenic effects in man and, hopefully, when coupled with the transplantation of carcinogenic treated tissues into nude mice, it will foster the development of entirely novel methods which will allow extrapolation of animal data to man.

Proposed Course: The future course will be to further characterize the organ culture characteristics of human bronchi and to extend the time in which organ culture is possible, hopefully, to several months. The morphological effects of chemical carcinogens on the bronchial epithelium will be studied. Moreover, the cultures will provide a means for studying inducibility of aryl hydrocarbon hydroxylase in human bronchi and for correlating the levels induced with those induced in other tissues such as lymphocytes.

Date Contract Initiated: October 1, 1973

Current Annual Level: $96,880

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NO1-CP-92083)

Title: Role of Vitamin A in the Control of Differentiation and Carcinogenesis in the Respiratory Tract

Contractor's Project Directors: Dr. George Wolf
Dr. Paul Newberne

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: The specific aims of this contract are the following: (1) To investigate the biochemical response, with respect to glycoprotein synthesis and secretion, of tracheal epithelium to vitamin A deficiency, vitamin A excess and to benzpyrene treatment, and their interactions; (2) To study the effect of vitamin A and benzpyrene and their interactions on the glycoproteins secreted by tracheas in organ culture; and (3) To investigate the histological

response of respiratory epithelium (in terms of tumor incidence, tumor type, etc.) to benzyprene carcinogenesis with retinol treatment, at different dose levels and times in hamsters fed semi-synthetic diets.

Major Findings: (1) Biochemical Work: (a) Glycoprotein Synthesis in Organ Culture. Systems were developed for culturing tracheas in a defined, vitamin A-free medium for 2 weeks, with maintenance of secretion of glycoprotein into the medium and active ciliary movement. Secreted glycoprotein was highly purified and characterized. Tracheas cultured from vitamin A-deficient hamsters showed a diminished uptake of labeled precursors into intracellular glycoprotein compared to those from normal hamsters; there was no observable effect of the deficiency on secreted glycoprotein, even after a high degree of purification. In vitro addition of 0.34 µg retinyl acetate to the medium per ml caused a significant decrease in intracellular glycoprotein synthesis after 6 days in culture, in tracheas both from deficient and normal hamsters.

Following a course of 10 instillations into hamster tracheas of benzpyrene-hematite in vivo (3x per week) with pyrene-hematite as control, tracheas were removed 2 and 6 weeks after the end of treatment and cultured for 6 days. After 2 weeks' recovery, uptake of labeled precursors into intracellular glycoprotein was reduced by 50%, whereas uptake into glycoprotein of the medium was stimulated to about the same extent. The glycoprotein was purified by fractionation, and the effect was primarily on one fraction. The effects after 6 weeks of recovery were less pronounced.

(b) Glycoprotein Synthesis after a 4-hour Incubation. In vivo instillations of BP and P-hematite were made as described above. The tracheas were removed, incubated with labeled fucose and glucosamine, epithelia were separated and their glycopeptides isolated and purified on DEAE-Sephadex. A fucose peak, eluted with 0.035 N LiCl, was slightly depressed 2 days after the instillations, depressed 32% 2 weeks after, and 58% 4 weeks after instillations. Glucosamine in the peak acted similarly. The affected glycopeptide was further purified on Sephadex G-50. Work is now in progress to establish a correlation to that glycopeptide of trachea which was found to be affected by vitamin A-deficiency.

(2) Animal Studies: Respiratory tract tumors were induced with benzo(a)-pyrene (BP)-Fe_2O_3 in groups of male Syrian hamsters fed either a semisynthetic (500 animals) or a chow (284 animals) diet. One half of the hamsters fed the semisynthetic diet were housed in laminar flow units and the other conventionally. Hamsters housed in laminar flow units developed respiratory tract tumors at a much slower rate following BP-Fe_2O_3 instillations than hamsters similarly treated and housed conventionally. Sixty percent of the hamsters in the laminar flow housing ultimately developed respiratory tract tumors while seventy percent of the hamsters in conventional housing developed respiratory tract tumors. Conventionally housed chow-fed hamsters had a respiratory tract tumor incidence of sixty-five percent.

Large doses of vitamin A did not significantly inhibit the formation of BP-induced respiratory tract tumors. The tumor incidence and spectrum was the same in groups of hamsters given either 50, 800 or 1200 µg of retinyl acetate twice a week per os, fed chow and housed conventionally. Hamsters housed

conventionally, fed the semisynthetic diet and in the highest vitamin A group had a slightly higher incidence of respiratory tract tumors while the hamsters in laminar flow housing, fed the semisynthetic diet and in the highest vitamin A group had a slightly lower respiratory tract tumor incidence. Retinyl acetate treatment was initiated one week following the last BP instillation and continued for the rest of the hamsters' lives. Serum and liver samples were monitored for vitamin A and these values corresponded with the oral retinyl acetate dosages.

Large doses of vitamin A did significantly inhibit the formation of papillomas of the forestomach in hamsters fed the semisynthetic diet and housed conventionally and in laminar flow units. Chow-fed, conventionally housed hamsters had a significantly lower incidence of papillomas of the forestomach compared to hamsters fed the semisynthetic diet and large doses of vitamin A did not inhibit papilloma formation.

Significance to Biomedical Research and the Program of the Institute: An understanding of the phenomenon whereby vitamin A inhibits respiratory carcinogenesis is of great significance to the problem of lung cancer in man. Evaluation of understanding of inhibition of carcinogenesis in the experimental animal is germane to an understanding of lung neoplasia in man and how vitamin A may help enhance differentiation of epithelium, the opposite of neoplasia. The optimal outcome of these studies will depend on how fully we can develop good animal models for the intelligent use of vitamin A or vitamin A analogs as anti-carcinogenic agents. These studies in turn will depend on a better understanding of the cellular and biochemical processes controlled by vitamin A. The present contract is immediately concerned with obtaining further information in the above areas.

Proposed Course: To continue the long-term animal studies already begun so that complete evaluation of the gross and microscopic pathology can be made. To continue with biochemical studies on the role of vitamin A in controlling normal differentiation of respiratory epithelium.

Date Contract Initiated: June 18, 1969

Current Annual Level: $228,173

MINNESOTA, UNIVERSITY OF (NO1-CP-33364)

Title: Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract

Contractor's Project Director: Dr. Lee W. Wattenberg

Project Officer (NCI): Dr. Carl E. Smith

Objectives: It has been demonstrated that several types of antioxidants will inhibit pulmonary neoplasia resulting from administration of polycyclic hydrocarbons. Specific objectives relevant to these observations are (1) to elucidate the mechanism of this inhibition, (2) to determine the conditions under which inhibition occurs, and (3) to ascertain optimal methods for utilizing antioxidants and related compounds to inhibit pulmonary neoplasia.

In a second part of the work, objectives are to determine the relationship between the activity and composition of the aryl hydrocarbon hydroxylase system and the binding of metabolites of polycyclic hydrocarbons to macromolecules of pulmonary structures, and in addition, to attempt to relate the amount of binding to the development of pulmonary neoplasia.

Major Findings: The present program is in its beginning phase so that few results are available on major aspects of the work. Some data relevant to the mechanism of BHA inhibition of polycyclic hydrocarbon induced neoplasia have been obtained. Studies of the affects of butylated hydroxyanisole (BHA) on binding of metabolites of benzo(a)pyrene (BP) to DNA in an *in vitro* system containing liver or lung microsomes have been carried out. In this experimental system a reduction in binding occurs. Most and possibly all of this decrease in binding is due to an inhibition of microsomal aryl hydrocarbon hydroxylase (AHH) activity by the BHA. In related studies efforts have been made to determine whether a direct reaction occurs between metabolites of 7,12-dimethylbenz(a)anthracene (DMBA) produced *in vitro* in a microsomal system and BHA. No such reaction product could be found nor does unaltered DMBA react with BHA. The findings in the experiments carried out thus far suggest that BHA is not inhibiting polycyclic hydrocarbon carcinogen induced neoplasia at the level of the ultimate carcinogenic species but probably acts by virtue of an effect on enzyme activity at a prior stage of carcinogen metabolism.

Significance to Biomedical Research and the Program of the Institute: Man now consumes a substantial amount of antioxidants. In animal models these compounds have been shown to have the capacity to inhibit chemical carcinogenesis. It is of importance to know the mechanism of this inhibition and to evaluate the present and potential role of antioxidants as inhibitors of the effect of chemical carcinogens in the environment on pulmonary neoplasia. Such information could be of importance in minimizing the impact of environmental carcinogens on pulmonary neoplasia in man.

Studies on the relationship between the activity and characteristics of the aryl hydrocarbon hydroxylase system and the development of pulmonary neoplasia comprise the second portion of the present project. The AHH system is subject to alteration in activity and characteristics by pure compounds as well as dietary constituents. It is of importance to determine how these alterations may affect the susceptibility of the host to neoplastic response from chemical carcinogens.

Proposed Course: The proposed course includes studies of the mechanism of antioxidant inhibition of polycyclic hydrocarbon carcinogen-induced pulmonary neoplasia. Accompanying animal studies will determine conditions under which neoplasia is inhibited and the relationships between chemical structure and capacity of compounds to exert inhibitory effects. Investigations are also in progress to determine the relationship between activity and characteristics of the AHH system and the extent of macromolecular binding of carcinogen metabolites to macromolecules and in addition, the relationship of these parameters to the occurrence of pulmonary neoplasia resulting from exposure to polycyclic hydrocarbon carcinogens.

Date Contract Initiated: June 23, 1973

Current Annual Level: $80,290

NEW YORK UNIVERSITY (NO1-CP-33260)

Title: Studies in Pulmonary Carcinogenesis

Contractor's Project Directors: Dr. Marvin Kuschner
Prof. Sidney Laskin

Project Officers (NCI): Dr. Carl Smith
Dr. David Kaufman

Objectives: The objectives of this contract have been the investigation of the mechanisms of pulmonary carcinogenesis and the examination of the carcinogenic potential of suspected materials. Its aims are to illuminate the process of malignant change as exemplified in the lung while, at the same time, providing immediate means for the prevention of lung cancer by the identification of hazardous materials. In particular, since it has become more and more evident that the induction of human lung cancer is multi-factorial in nature and dependent upon long-term, low level exposures to combined agents, one objective of this contract is to evaluate this combined action in a wide variety of animal models using a variety of experimental techniques.

Major Findings: Animal models have been developed which duplicate human bronchogenic carcinoma. The production of such tumors has been achieved by a variety of techniques which include thread transfixion, pellet implantation, direct intrabronchial instillation, and finally, inhalation. Dose-response relationships have been developed from the intrabronchial pellet technique with polycyclic hydrocarbons.

An extensive series of studies are being performed in this laboratory to study the effects of several components of air pollution. This laboratory was the first to produce lung cancer in rats with combined inhalation exposures to sulfur dioxide and benzpyrene. Studies with combined carcinogen-irritant exposures are proceeding in an effort to reinforce the original results. Findings to date confirm the original studies.

In order to define the progression of changes which result in cancer, an extensive dose-response study using hamsters and intratracheal intubations of methylcholanthrene was performed. Results showed an excellent dose-response relationship, a shortening of the time needed to develop cancer as dose increases, and cancer incidence approaching 100% at 24 intubations of 1 mg methylcholanthrene.

The hamster intubation technique has also provided a valuable model for carcinogen-irritant studies. Animals intubated with carcinogens are currently being exposed by inhalation to sulfur dioxide atmospheres. Early results have shown findings of adenocarcinoma in animals given 10 intubations of methyl-

cholanthrene and lifetime exposures to sulfur dioxide. Benzpyrene, which has shown negative results by intubation only, in combination with exposures to sulfur dioxide has shown several carcinomas.

Short term and chronic inhalation studies with nitrogen dioxide, a component of air pollution and cigarette smoke, are complete. Although mucosal changes after exposures to 25 ppm appear to be minimal, endothelial damage has been reported with electron microscopy. Later results suggest a low incidence of panacinar emphysema, particularly in the hamster.

Studies have been initiated for combined exposures of rats to nitrogen dioxide and benzpyrene. A similar study with hamsters will be initiated immediately following completion of the study currently utilizing the second isolation unit.

Experiments with tobacco tar exposure by means of intrabronchial pellet implants with large numbers of animals have been performed. Test materials in cholesterol carrier include benzpyrene alone, tobacco tar alone, and mixtures of the two. Results, to date, suggest specific effects of the acid washed tobacco tar. These include a squamous cell carcinoma of the trachea and a malignant tumor of undertermined type and origin in the lung.

Preliminary inhalation studies with croton oil have been completed. Studies involving carcinogen intubations with subsequent croton oil inhalation exposures are underway. Techniques employed involve the use of single or multiple intubations in hamsters with methylcholanthrene or benzpyrene followed by inhalation of croton oil.

Compounds of chromium have been demonstrated to be capable of producing bronchogenic carcinoma of the lung in experimental animals. The identification of calcium chromate as one of the major agents in the chromate problem has resulted in inhalation and clearance studies with this compound. The recently completed chronic study at 2 mg/cu m has produced carcinomas in rats and hamsters.

Studies are underway with current industrial chrome process materials provided by the industry. Since a survey of the industry and evaluation of experimental results implicates process residue as the major potential agent, a chronic inhalation study has been started with this material.

Two industrial plastic dusts were studied to determine their possible effects on workers in the industry. Reinforced polyester-fiberglass plant dust was studied utilizing the intubation technique and short-term inhalation exposures. Centrilobular emphysema and other suggestive changes of interest were noted. With polyurethane foam, animals were given 30 inhalation exposures to freshly generated foam dust atmospheres at 2 levels. Centrilobular emphysema and squamous cell carcinoma were seen in rats at both levels.

In view of the large population of construction workers involved in the use of such materials, chronic studies are being developed for the polyurethanes. Preliminary concentration and particle-size tests are in progress with current samples of the most commonly used polyurethanes. For these studies, a newly designed dust feed system has been developed. Additionally, a new sampling

instrument for particle-size evaluation has also been developed.

Inhalation studies were completed with two chlorinated ethers used widely as industrial reaction intermediates. Both compounds proved to be carcinogens. The bis(chloromethyl)ether showed extremely high incidences of carcinoma at 0.1 ppm. The extremely low levels and the magnitude of the carcinoma response with bis(chloromethyl)ether suggested a very serious new industrial hazard. This was recently reinforced by the publication of a report documenting 14 human cases in exposed workers (New England Journal of Medicine, May 24, 1973). These compounds are now listed in the OSHA carcinogen regulations. Recently, preliminary studies in this laboratory suggest that hydrochloric acid and formaldehyde, at levels approximating industrial exposures, can produce bis-(chloromethyl)ether. The widespread use of such materials indicates the need for animal inhalation studies.

Results with chloromethyl methyl ether and bis(chloromethyl)ether have prompted examination of a variety of other alkylating agents of industrial importance. Preliminary studies are underway with the chlorinated ether dimethylcarbamyl chloride.

Significance to Biomedical Research and the Program of the Institute: This contract represents a component of a broad effort to develop bioassay techniques for studies on lung cancer. It is also important for its study of potential hazards of materials which are in use industrially and may be found in the environment.

Proposed Course: To extend previous studies and develop inhalation studies with other types of suspected materials.

Date Contract Initiated: June 29, 1966

Current Annual Level: $456,000 (Estimated)

OHIO STATE RESEARCH FOUNDATION (NO1-CP-92144)

Title: Study of the Role of Vehicles and Particulates in Respiratory Carcinogenesis Bioassay

Contractor's Project Director: Dr. Robert Farrell

Project Officer (NCI): Dr. David G. Kaufman

Objectives: To determine the effect of intratracheal instillation of fine particulates of varied chemical and physical properties and of various types of solvents on the induction of carcinoma of the lung by chemical carcinogens.

Major Findings: A long-term carcinogenesis experiment to examine the effects of Tween 80 given together with benzo(a)pyrene (BP) via intratracheal instillation in a gelatin-saline vehicle has been completed. Results suggest that animals treated with Tween 80 in addition to BP may develop slightly fewer malignant respiratory tract tumors than do those animals treated with BP alone.

For another group of experiments, chemically and physically defined fine particles, both coated with BP, and uncoated, have been produced by another contract (IIT Research Institute - NIH-NCI-E-33296). Long term carcinogenesis studies were performed to determine and compare the carcinogenicity of these preparations. A high incidence of squamous carcinomas was induced in the trachea and bronchi with BP adsorbed to Fe_2O_3 and carbon particles. There were 89 tumor-bearing animals (TBA) in the BP-Fe_2O_3 groups, 14 TBA in the BP - Al_2O_3 groups and 119 TBA in the carbon groups.

Long term carcinogenesis studies to determine the effect of uncoated particles of different sizes and composition, administered via intra-tracheal instillation (IT), on the induction of respiratory tumors by diethylnitrosamine (DEN) have been completed. Animals treated with DEN and IT particulates developed more tumors (benign and malignant) of the nasal cavity than did animals treated with DEN only (benign tumors only).

Significance to Biomedical Research and the Program of the Institute: This project is one of several designed to develop techniques for studying the most common type of lung cancer found in man. It is based on previous observations that epidermoid carcinomas of the lung could be induced in hamsters by the intratracheal administration of iron oxide dusts along with the intratracheal administration of benzo(a)pyrene. It was observed that there was a difference in the incidence of bronchogenic carcinomas between the types of particles used for vehicles. This may relate to different types of industrial particulate pollution and, in the presence of carcinogens, to their effects on the induction of bronchogenic carcinoma in man.

Proposed Course: The contract under which this work was done has terminated.

Date Contract Initiated: June 27, 1969

Current Annual Level: None

ST. MARY'S HOSPITAL (NO1-CP-23233)

Title: Morphogenesis of Lung Cancer

Contractor's Project Director: Dr. Geno Saccomanno

Project Officers (NCI): Dr. Norbert P. Page
Dr. Carl E. Smith

Objectives: The objective of this contract is to learn about the morphogenesis of human lung cancer by sputum cytological analysis, with the correlation of cytology and histology of lung cancer.

Major Findings: In 1973, 2,252 sputum samples from uranium miners were collected, slides prepared, reported, and filed. Five new cases of carcinoma have been identified. Nineteen cancer of the lung deaths occurred among the uranium miner population. Twenty-six autopsies were performed, and these will be studied to collaborate cytological findings. Carcino-embryonic

studies of the miners reveal a correlation with neoplasia and squamous cell metaplasia. Approximately 2,000 sputum aspirates on hamster cancer studies were read and reported in collaboration with Dr. Paul Nettesheim (AEC-Oakridge National Laboratories).

Significance to Biomedical Research and the Program of the Institute: Identification of early stages in the morphogenesis of human lung cancer and the determination of the characteristics of this high risk population are of vital importance. Experimental study applicable to these goals in the hamster model is similarly relevant.

Proposed Course: This project will continue to collect, process, and analyze sputa from uranium miners. As many autopsies as become available from this population will be performed and processed to correlate cytological specimens and tissue sections.

Date Contract Initiated: May 1, 1972

Current Annual Level: $152,399

SOUTHERN RESEARCH (NO1-CP-22064)

Title: Organ Culture and Tissue Culture Bioassay of Vitamin A Analogs

Contractor's Project Directors: Dr. Lee Wilkoff
Dr. Donald Hill

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: Vitamin A has been shown to have an anticarcinogenic effect in several different test systems involving whole animals. The utility of vitamin A as an anticarcinogenic agent is limited by undesirable toxic side effects which result from high doses of this material. The Carcinogenesis Program is procuring new analogs of vitamin A, which are intended to have a higher degree of selectivity, that is, a higher ratio of anticarcinogenic effects with respect to undesirable toxic side effects. The objectives of the present contract are (1) to develop adequate bioassays to evaluate the biological activity of these new analogs (as measured by their control of epithelial differentiation); and (2) to perform bioassays on selected analogs.

At present, there are no defined quantitative bioassays for vitamin A activity in organ culture or tissue culture. Consequently, the primary objective of this contract is to develop new assays for vitamin A activity on a quantitative basis.

Major Findings: Organ culture of metatarsal skin from 13-day-old chick embryos has been developed. Explants cultured in BGJ medium + 5% fetal calf serum consistently keratinize in six days with moderate to abundant keratin present at eight days in culture. Explants cultured in the presence of effective concentrations of all-trans-retinoic acid, all-trans-retinyl acetate or all-trans-retinol exhibit a mucous metaplasia in six to eight days. In addition

to explants that exhibited a squamous keratinizing metaplasia or a mucous metaplasia, two other histological states have been observed when relatively low concentrations of effective vitamin A analogs are used. At certain concentrations, explants exhibit (a) areas of keratinization and areas of mucous metaplasia within the same explant and/or (b) no histological evidence of keratin or mucous formation. These two states may represent intermediate responses of the explants to analogs at relatively low concentrations. This system is being used to monitor for specific biological properties of vitamin A analogs: the inhibition of keratinization and the production of mucus. The effectiveness of the following analogs (listed in order of decreasing activity) have been evaluated in the system; cyclopentene derivative of retinoic acid > retinoic acid methyl ester > retinoic acid ethyl ester = retinoic acid ethyl amide = α-retinoic acid = 13-cis-retinoic acid > all-trans-retinoic acid > all-trans-retinol >retinoic acid diethylamide > α-retinyl acetate.

A gas-liquid chromatographic assay for mucos production, based on the sugar content of explants, indicates that explants exhibiting a mucous metaplasia have a two- to threefold increase in fucose content.

The aldehyde analogs of vitamin A are the most toxic, and retinyl palmitate and the cyclopentene derivative of retinoic acid the least toxic, to KB cells in culture. Mouse fibroblast cells are significantly more sensitive to vitamin A compounds than are KB cells. Release of acid phosphatase, β-glucuronidase, and deoxyribonuclease from mouse liver lysosomes by vitamin A was measured. Most effective are retinyl acetate, retinol, 13-cis-retinol,N, N-dimethyl retinoate, and 13-cis-retinoate. A good correlation exists between toxicity of these compounds to mouse fibroblast cells and the release of β-glucuronidase or deoxyribonuclease.

The vitamin A compounds have been evaluated as inhibitors of microsomal mixed function oxidases from liver and lung tissues of mice and hamsters. Some of the compounds are potent inhibitors of the enzymatic oxidation of benzo(a)-pyrene and other carcinogenic, polycyclic hydrocarbons to products which become tightly bound to reaction components. The hydroxylation of benzo(a)-pyrene to the 3-hydroxy derivative is not inhibited, but the further metabolism of 3-hydroxy-benzo(a)pyrene is blocked. Alterations in the metabolism of polycyclic hydrocarbons by vitamin A compounds may be related to their anticarcinogenic action.

Significance to Biomedical Research and the Program of the Institute:
Vitamin A is definitely known to be required for the normal differentiation and growth of the epithelium in the following organs, all of which are major sites of primary cancer in man: bronchi and trachea, colon, stomach, uterus, kidney, and bladder, testis, and skin. The role of vitamin A in controlling normal differentiation and growth in tracheobronchial and intestinal epithelium is currently under intensive investigation in the Lung Cancer Unit, Office of the Associate Scientific Director for Carcinogenesis.

In addition to controlling the differentiation of many types of normal epithelium, vitamin A is also able to alter the process of chemical carcinogenesis in several different epithelia and to block the effect of several different carcinogens. Thus, it has been found that vitamin A can block the

carcinogenic effects of polycyclic hydrocarbons on tracheo-bronchial, gastric, and uterine (cervical) epithelium. In some of these studies, it has been shown that the effect of vitamin A may not be due to alterations of the immediate metabolism of the carcinogen, since vitamin A is able to inhibit carcinogenesis even when administered several weeks after the carcinogen. Studies are currently in progress in the Lung Cancer Branch, Carcinogenesis Program, which are attempting to define the biochemical and cellular role of vitamin A in blocking epithelial carcinogenesis. Presumably, this effect is directly related to the biochemical and cellular role of vitamin A in determining normal differentiation in these tissues, but the proof of this is not yet at hand.

The ultimate implications of these studies on the inhibition of epithelial carcinogenesis are very important. If vitamin A is indeed functioning as a differentiation hormone in this situation, then it should be possible, with the skill and cooperation of a group of organic chemists, to design and synthesize new analogs of vitamin A which will have even greater potency than vitamin A itself on differentiation of epithelium. The precedents in steroid chemistry for this approach to vitamin A are very impressive; presently there are many synthetic steroid analogs which are much more potent (and often more specific and less toxic in action) than the naturally occurring estrogens, progestational agents, androgens, or adrenocortical steroids. If this type of approach could be applied to the hormonal action of vitamin A, then the potential for inhibiting carcinogenesis in many types of epithelia would be greatly increased.

Proposed Course: Continue the development of bioassay methods using organ culture systems to evaluate the biological activity of vitamin A analogs. Devise new methods, using morphological, histochemical, and biochemical end points for quantitative bioassay of vitamin A and vitamin A analogs. Determine the toxicities of vitamin A analogs to both KB (epithelial-like) and fibroblastic cell lines.

Measure the inhibition by vitamin A compounds and analogs of microsomal oxidases of liver and epithelial tissues which are responsible for metabolism of polycyclic hydrocarbons. Investigate the possibility that vitamin A must be converted to a biologically active form before it influences differentiation of epithelial tissue.

Date Contract Initiated: December 10, 1972

Current Annual Level: $185,000

STANFORD UNIVERSITY SCHOOL OF MEDICINE (NO1-CP-43295)

Title: Studies on Oat Cell Carcinoma of the Lung

Contractor's Project Director: Dr. Klaus G. Bensch

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: Development of a reliable means for identification of the bronchial counterpart of the intestinal argentaffine (Kulchitsky) cells (K-cells), their distribution in the human lung, their adaptation to organ and cell culture, and elucidation of factors in their neoplastic transformation.

Major Findings: It is extremely difficult to stain for light microscopy the bronchial K-cells in a reproducible, predictable way; a very large variety of stains, modes of fixation and histochemical techniques was tested for this purpose. Survival of K-cells in explants and organ cultures of human mammalian bronchi was limited. Over ten oat cell tumors were used to establish tissue culture cell lines. These are being screened for the presence of neurosecretory granules and specific compounds, including embryonal proteins.

Significance to Biomedical Research and the Program of the Institute: Oat cell carcinomas of the lung arise from a specific type of cell of the human respiratory tract. Although these cells appear to be present as a relatively small proportion of the population of the bronchial and bronchiolar lining, the incidence of this type of tumor is very high in males. These studies should shed light on the distribution of these cells in human bronchi, and experiments with *in vitro* cultures of these cells are aimed at identification of factors, or of mechanisms, active in the neoplastic transformation of these cells.

Proposed Course: Development of means for ready identification of K-cells, including the use of fluorescent antibody techniques; distribution, replication and growth pattern of these cells; isolation and identification of specific secretory products; uptake by these cells of compounds known to be associated with high incidence of oat cell tumors and their effect on cell growth and replication.

Date Contract Initiated: September 4, 1972

Current Annual Level: $79,287

STATE UNIVERSITY OF NEW YORK (at Stony Brook) (NO1-CP-33361)

Title: Studies of Carcinogenesis in Organ Culture of Trachea and Bronchi

Contractor's Project Director: Dr. Bernard Lane

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: The objectives of this contract are the following: (1) to determine the methodology for testing for biologic (*in vivo*) malignancy of tracheal epithelium transformed in organ culture, (2) to determine the optimal conditions for the organ culture of tracheal rings, (3) to observe the biology of tracheal ring cultures, (4) to determine the effects of exposing tracheal rings to the carcinogen benzo(a)pyrene (BP) *in vitro* and (5) to monitor cultures exposed *in vitro* for malignant transformation.

Major Findings: On the basis of *in vivo* experience and *in vitro* pilot experiments, the rat has been selected as a suitable species for the study of benzo(a)pyrene induced malignant transformation of tracheal epithelium in organ cultures. An inbred strain of rats (Wistar-Lewis) has been selected which will permit tests for biologically malignant behavior involving implantation of the transformed tissue in a syngeneic host. Using tissues from the inbred strain, tracheal rings grown in culture for two to three weeks and then implanted subcutaneously or intraperitoneally for one to five weeks showed no histologic evidence of rejection although there is in transplants between members of an outbred strain. Subcutaneous implantations show some fibrosis of the outer surface of the ring but the inner surface forms a pouch lined by epithelium exhibiting normal histologic and cytologic appearance. The outer surface is not maintained even if the rings are implanted into a previously formed fibrous pocket under the skin. Some intraperitoneal implants do retain the epithelium on the outer surface of the ring. However, the subcutaneous site will be used in most future work because of the ease with which rings are recovered.

A number of studies concerning effects of culture conditions on growth of tracheal epithelium have been completed. McCoy's 5a medium will support growth of the epithelium and ambient oxygen tension is sufficient for long term viability of ring cultures in the presence of 5% CO_2. The rings suffer morphobiological damage at low pH that is probably irreversible, but they tolerate pH 6.8 indefinitely. Vitamin A in the culture medium at a concentration of 0.1µg/ml aids in maintaining epithelial specialization. Higher concentrations are toxic while lower concentrations are ineffective. The cultures can be grown with or without serum.

The biology of rings in culture has been studied using light and electron microscopy and autoradiography. When the tracheal rings are carefully cut with a custom made slicing device, the inner epithelial surface suffers little damage and remains intact. After four days in culture, both the inner and outer surfaces of the rings are covered with epithelium. Growth of the epithelium is shown by mitotic activity which is observed initially on the inner surface of the rings (the original mucosal surface) and by day five, one in ten cells on the outer surface of the ring incorporates radioactive thymidine given as a 0.5 hr pulse. By day 15, mitotic activity is virtually nil. Electron micrographs of the basal lamina show that it is focally lost on the inner surface and focally formed on the outer surface. The culture medium with calf serum maintained differentiation of the epithelium for at least six months as shown by the presence of beating cilia. Micrographs of Goblet cells in long-term cultures show retention of granular endoplasmic reticulum and Golgi apparatus without secretory droplets. Basal cells are also recognizable.

Changes in the growth of the epithelium have been observed following the addition of the carcinogen BP to the culture medium. Varying the concentration of BP shows that exposure to 0.5µg/ml induces hyperplasia also while exposure for 4 weeks at 2µg/ml or for two months at 1µg/ml produces atypia. BP is toxic to ring cultures at 4µg/ml while 1µg/ml allows indefinite survival. Autoradiography of sections from rings cultured in 2µg/ml BP shows DNA synthesis in 20 to 30% of cells while colchicine arrest of such cultures shows mitosis in 5-10% of cells observed.

Preliminary observations have been made in the effort to monitor in vitro transformation of epithelial cells. Cytologic evidences show early nuclear atypia with a high N:C ratio. The nuclei are larger than those observed during regeneration. Histologic evidence of disordered growth during culture in BP includes nodule formation with some downward spikes. Karyotypic analysis of transformed cells has proved feasible: mitotic figures can be recognized in squash preparations of epithelium obtained directly from the cultured rings.

Significance to Biomedical Research and the Program of the Institute: The tracheobronchial epithelium gives rise to more fatal human tumors than any other tissue. Yet experimental access to many parameters of carcinogenesis under controlled conditions, including dependable tests of malignant transformation, has been limited. Long-term organ culture of tracheobronchial epithelium constitutes a model which preserves the biologic organization of the tissue while permitting control of almost all variables affecting population kinetics and cellular differentiation, stages in carcinogenesis and modification of carcinogen effects by other agents. This contract, and a companion contract to it, is directly concerned with the problems of determination of causes of lung cancer, with the early diagnosis of lung cancer lesions, and with effective prevention of lung cancer.

Proposed Course: The proposed course is to transplant rings which have been cultured in BP, to monitor the hosts for metastasis and to examine recovered transplanted rings and the surrounding tissue for invasive growth. At the same time, methods for monitoring early transformation in vitro including ultrastructural changes, binding of carcinogen in the target cell, karyotype differences, changes in the phases and timing of cell cycles, the appearance of new antigens, deletional mutations observed by histochemical, biochemical or immunological techniques, and changes in sensitivity to mitogens are to be studied. In addition, a technique for culturing a pure population of epithelial cells will be sought in order to facilitate some of these studies, especially those involving biochemical changes.

Date Contract Initiated: June 25, 1973

Current Annual Level: $123,300

STOCKHOLM, UNIVERSITY OF (NO1-CP-33363)

Title: Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract

Contractor's Project Director: Dr. Lars Ernster

Project Officers (NCI): Dr. Carl E. Smith
Dr. David G. Kaufman

Objectives: The objective of this contract is to define the relation between the enzyme system, aryl hydrocarbon hydroxylase, and pulmonary neoplasia. Specific aims are (1) determination of the distribution of substrates and products of aryl hydrocarbon hydroxylase in pulmonary tissue, (2) subcellar fractionation of lung cells and subcellar localization of aryl hydrocarbon

hydroxylase activity, (3) employment or development of a highly sensitive assay for aryl hydrocarbon hydroxylase, including studies of tissue disruption to optimize enzymatic activity accessible to assay, (4) dissociation of the cells of the lung and isolation of individual cell types, (5) characterization of the properties of lung aryl hydrocarbon hydroxylase, including identification of polycyclic hydrocarbon metabolites in pulmonary tissues, and (6) initial studies with human lung.

Major Findings: Studies of polycyclic hydrocarbon metabolism in the respiratory tract have been proceeding along two lines. On one hand have been efforts to develop a better assay for aryl hydrocarbon hydroxylase activity, while at the same time subfractionation experiments have begun with rat lung designed to yield a microsomal preparation consisting chiefly of fragments of the endoplasmic reticulum.

The standard fluorescent assay for aryl hydrocarbon hydroxylase has a number of shortcomings. It detects only some of the products of the enzyme system; it is unsuitable for use with tissue homogenates or sonicates, for some as yet unexplained reason, and despite its great sensitivity, it is not sensitive enough for certain important kinds of studies. Further, the standard fluorescent assay for aryl hydrocarbon hydroxylase is technically difficult to carry out and time-consuming.

An assay has been developed based on the use of ^{3}H-benzpyrene as substrate. The incubation is stopped with an aliquot of sodium hydroxide dissolved in ethanol; all of the unreacted benzyprene is extracted into hexane, leaving all of the products in the water phase; and an aliquot of the water phase is then counted. Thin layer chromatography indicates that indeed only unreacted substrate is extracted into the hexane; controls show that the background in the assay, i.e., the amount of unreacted substrate that remains in the water phase, is about 0.5%. This assay is linear with time for at least 30 minutes, and with protein concentration at least over the range of 0.2-1.0 mg microsomal protein. Use of ^{14}C-benzpyrene gives results identical to those obtained with ^{3}H-isotope. The classic inhibitors of the mixed function oxidase - e.g., anaerobiosis, cytochrome c. and carbon monoxide - strongly inhibit benzpyrene hydroxylase as measured by this assay. The assay can be carried out rapidly and can be made quite sensitive by scaling it down.

In parallel with the above experiments, studies have been made on the homogenization and fractionation of rat lung tissue resulting in a number of interesting observations. Clamping off the vena cava and perfusing the lung *in situ* with physiological saline solution through the right chamber of the heart removes a great deal of hemoglobin which would otherwise contaminate microsomal preparations and interfere with spectroscopic studies. If the rat lung is homogenized at a "concentration" of 2.5 grams per 10 ml 0.25 M sucrose, the homogenate often becomes very viscous and gel-like; the microsomal fraction (prepared as with liver) contains only 28% of the total tissue NADPH-cytochrome c reductase activity (used as a marker for the endoplasmic reticulum) at a specific activity 4.5 times that of the whole homogenate; and the microsomes are contaminated with 19% of the total succinate-cytochrome c reductase (a marker for mitochondria). On the other hand, if homogenization is carried out at a "concentration" of 1.0 grams per 10 ml 0.25 M sucrose,

the formation of a gel is avoided; the microsomal fraction contains 55% of the NADPH-cytochrome c reductase at a specific activity 7.6 times that of the whole homogenate; and the contamination of the microsomes with mitochondria is reduced 2.5 fold. We find very little glucose-6-phosphatase in the rat lung, so that this activity is not suited for use as a marker for the endoplasmic reticulum in these studies. Preliminary induction studies reveal that treatment of rats with methylcholanthrene induces cytoplasmic DT diaphorase, microsomal DT diaphorase, cytochrome P450, aryl hydrocarbon hydroxylase, IDPase, and ATPase, but not NADPH-cytochrome c reductase or cytochrome b_5 in rat lung microsomes.

Finally, arrangements have been made for access to human lung tissue on a regular basis and preliminary preparative experiments performed.

Significance to Biomedical Research and the Program of the Institute: Many of the foreign compounds which enter the human body do so through the lungs. Among these are the polycyclic hydrocarbons which may be a major cause of human cancer. The principal pathway for metabolism of the polycyclic hydrocarbons in mammalian tissues is the inducible enzyme system aryl hydrocarbon hydroxylase. This contract and others in the program are directed towards a detailed understanding of the nature and function of this enzyme system and its relationship to carcinogenesis in the respiratory tract.

Proposed Course: Having now developed a sensitive and reliable radioactive assay for benzyprene hydroxylase, this assay will be employed in (a) further subcellular fractionation studies on rat lung; (b) investigations of the properties of benzpyrene hydroxylase in rat lung; and (c) investigations of the properties of benzpyrene hydroxylase in human lung tissue.

Date Contract Initiated: June 25, 1973

Current Annual Level: $114,753

TEXAS, UNIVERSITY OF (Southwestern Medical School at Dallas) (NO1-CP-33362)

Title: Studies on Polycyclic Hydrocarbon Metabolism in the Respiratory Tract

Contractor's Project Director: Dr. Ronald Estabrook

Project Officer: Dr. Carl E. Smith

Objectives: The objective of this contract is to define the relation between the enzyme system, aryl hydrocarbon hydroxylase, and pulmonary neoplasia. Specific aims are (1) separation and isolation of the microsomal fraction of lung tissue from unexposed and polycyclic hydrocarbon-induced animals, (2) physicochemical and enzymatic characterization of the isolated cell fractions, including the cytochrome P-450 and P-448, and the isolation of these components, and (3) complementary studies on the isolation of NADPH-cytochrome P-450 reductase from microsomes of lung tissue.

Major Findings: Using normal methods of differential centrifugation, a satisfactory method for preparation of microsomal fractions of hamster and rat

lung has been developed. The microsomal cytochromes, cytochrome P-450, and cytochrome b_5, have been studied in control and intraperitoneal polycyclic hydrocarbon treated animals. Hemoglobin and methemoglobin exist as major contaminants in lung microsomes and accounts for a major portion of heme proteins which absorb near the 420 nm region of the visible spectrum. The concentration of these cytochromes are in the order of one thirtieth (1/30) of the concentration of the corresponding cytochromes in uninduced liver. The following physicochemical studies are in progress on the nature of the lung cytochromes: ultraviolet spectroscopy, low-temperature spectroscopy, and rapid-kinetic analysis of cytochrome reduction by physiological substrates.

The cytochrome P-450 (448)-dependent enzyme systems of lung have been studied during control and intraperitoneal polycyclic hydrocarbon treatment. This system is extremely responsive to such carcinogen treatment and responds, in general, in a manner similar to the liver cytochrome P-450 (448) system. However, differences exist between liver and lung induction using phenobarbital as an inducing agent. Phenobarbital appears to be a poor inducing agent for the lung cytochrome P-450 (448) system. The enzyme activities of lung microsomes from hamster and rat are induced from low levels in control lung to levels nearly as high as that of liver in carcinogen treated rodent lung.

Purification studies on several components of lung microsomes are in progress, but due to the low concentrations of the lung cytochromes the studies are preliminary. The NADH and NADPH cytochrome c reductases of lung are being compared to the corresponding liver reductases (NADH cytochrome b_5 reductase and NADH cytochrome c reductase, respectively). Both activities in lung have specific activities (nmoles cytochrome c reduced per minute per mg protein) which are one-third of those reported in liver.

Significance to Biomedical Research and the Program of the Institute: Polycyclic hydrocarbons and their metabolites are recognized as potential carcinogenic agents. A major route of introduction of polycyclic hydrocarbons into the human is through the respiratory tract. The primary mechanism of metabolism of polycyclic hydrocarbons involved oxygen activation by a unique hemoprotein-containing electron transport system associated with the endoplasmic reticulum of many tissues. This contract and others in the program are directed toward a detailed understanding of this enzymatic system functional in the oxidative metabolism of polycyclic hydrocarbonss and its role in respiratory tract carconogenesis.

Proposed Course: The current biochemical characterization of lung microsomal fractions will be continued to complete the work described in the findings section. The purification studies will also be continued, but a major emphasis will be on the metabolism of aromatic hydrocarbons in lung. Since the role of metabolism of polycyclic hydrocarbons in lung neoplasia is not well understood, the study of polycyclic hydrocarbon metabolism and of the factors involved in polycyclic hydrocarbon metabolism is imperative. Preliminary studies on alternate assays of arylhydrocarbon hydroxylase with non-carcinogenic substrates are in progress.

Date Contract Initiated: June 20, 1973

Current Annual Level: $97,696.67

VERMONT, UNIVERSITY OF (NO1-CP-33360)

Title: Studies of Carcinogenesis in Organ Culture of Trachea and Bronchi

Contractor's Project Director: Dr. John E. Craighead

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: This project has five basic objectives: (1) the development of organ culture systems which permit viability and differentiation of hamster tracheal epithelium for extended periods of time, (2) the development of techniques for the application of carcinogens quantitatively to respiratory tract epithelium in organ culture, (3) the development of techniques for the transplantation of organ culture tissue into susceptible host to document *in vivo* neoplastic transformation due to carcinogen exposure *in vitro*, (4) an assessment of the carcinogenic properties of selected carcinogens in differentiated respiratory epithelium, and (5) the detection of possible type C virus activation in animal tissue treated with carcinogen *in vitro*.

Major Findings: Viable, differentiated hamster tracheal epithelium is currently maintained in organ culture in medium containing low concentrations of serum for two or more months as confirmed by histologic study and radioautography. A systematic evaluation of several chemically-defined media has shown that some encourage active epithelial proliferation, whereas others promote the growth of a nonciliated cuboidal epithelium. Serumless media produce disorganized epithelial changes which resemble squamous metaplasia.

Borosilicate glass fibers (diameter 3.10^{-2}) have been dipped in various concentrations of radiolabeled 3-methylcholanthrene in acetone and applied (after evaporation of the acetone) to the epithelial surface of the organ cultures. This technique permits us to vary the amount of carcinogen applied to the epithelium and allows a systematic evaluation of epithelial changes at a defined site over a range of time periods. Detailed studies have shown that the concentration of hydrocarbon on the fiber can be accurately regulated.

Preliminary observations indicate that epithelial cells exhibit cytologic alterations and changes in ^{3}H-thymidine uptake at sites of fiber application after brief periods of exposure. Proliferation of the affected mucosa and loss of orientation of epithelial cells is noted.

Tracheal rings maintained in organ culture for one month and longer have been implanted into subcutaneous sites and into cheek pouches of syngeneic animals for periods of as long as 3 weeks. Implants exhibit excellent epithelial and ciliary preservation with no invasion by inflammatory cells. Carcinogen-treated cultures recently have been implanted into animals. These tissues will be examined at regular intervals for evidence of neoplastic growth.

Significance to Biomedical Research and the Program of the Institute: The association of bronchogenic carcinoma with cigarette use is well established on epidemiologic grounds. Although a number of cigarette smoke and tar constituents have been identified as probable carcinogens or co-carcinogens, their mode of action upon the tracheal-bronchial tree remains undefined.

A number of studies have explored the effects of polycyclic hydrocarbons on epithelial cells and fibroblasts in monolayer culture, however, since cells in monolayer are relatively undifferentiated, this approach does not provide definitive information on the carcinogenic effects of inhalants on the differentiated epithelial cells of the respiratory tract. Maintaining the differentiated respiratory epithelium in organ culture for extended periods of time allows a systematic evaluation of carcinogenic agents and their mechanism of interaction with the differentiated respiratory epithelial cell.

Proposed Course: Long-term culture experiments examining serum and vitamin A effects on tracheal epithelium are currently in progress. Selected tissues at monthly intervals will be fixed in glutaraldehyde and osmium tetroxide and prepared as sections in an ultramicrotome for improved radioautography and better histologic definition. Selected carcinogen-treated tissue will also be evaluated ultrastructurally to better define cytologic alterations.

Benzo(a)pyrene and other test compounds will be employed over a range of concentrations as test carcinogens in the proposed experimental system. Animal tumors induced by carcinogen-treated specimens grown in organ culture will be maintained in serial passage in syngeneic hosts until their growth properties and histologic features are fully assessed.

Specific tests will be carried out at critical junctures in the course of this investigation to assess for activity of type C viruses in the organ cultures and host animals with transplanted tumors.

Date Contract Initiated: June 28, 1973

Current Annual Level: $97,978

VETERANS ADMINISTRATION HOSPITAL (TAMPA, FLORIDA) (YO1-CP-40204)

Title: Autoradiographic Study of the Cellular Response of the Respiratory Tract in Chemical Carcinogenesis

Contractor's Project Director: Dr. Hollis Boren

Project Officer (NCI): Dr. Curtis Harris

Objectives: The objective of this project is to determine the proliferative cellular response of various segments of the respiratory tract following exposure to respiratory carcinogens and particulates, using primarily the hamster intratracheal instillation model which is studied in other respects in several other contracts. These measurements are correlated with the histopathological response to carcinogens and particulates. In addition, radioactive carcinogens and related compounds will be localized in the respiratory epithelium by quantitative light microscopic autoradiography.

Major Findings: Hamsters were maintained on vitamin A free gelatin diet supplemented with 75 µgm retinyl acetate twice weekly, After seven weeks they were given 5 weekly intratracheal (i.t.) instillations of 3 mg benzo(a)pyrene and 3 mg ferric oxide (FO), then were divided into three groups to receive

75 μgm, 225 μgm or 675 μgm retinyl acetate biweekly. Animals were sacrificed before receiving any carcinogen and after carcinogen at 10-week intervals for determination of liver vitamin A levels, length of DNA synthesis (double labeling with ^{3}H- and ^{14}C-thymidine) and labeling index (colchicine and continuous labeling with ^{3}H-thymidine). In a collaborative study, ^{3}H-thymidine was given 1, 2, 4, 7, or 10 days or 2, 3, 4, or 8 weeks following a single i.t. dose of 1, 3, or 5 mg BP plus 1, 3, or 5 mg FO; 1, 3, or 5 mg FO; or sham treatment. Early results indicate that either BP + FO alone increases the number of cells which incorporate ^{3}H-thymidine. BP seems to diminish the number of heavily labeled cells. Quantitative autoradiography is used for studies of cellular kinetics and intracellular binding. A method of simultaneous determination of latent image fading and emulsion sensitivity revealed no latent image fading and unchanged emulsion sensitivity for six months.

Significance to Biomedical Research and the Program of the Institute: This contract is an integral part of an interlocking group of contracts designed to define the role of carcinogens and the physico-chemical factors (e.g., particulates) required for respiratory carcinogenesis. The other studies, including long-term tests, have been designed so that they are closely related to the present one with maximum interaction but no overlap of activity, in an effort to define the key role of vehicles and particulates in the process of respiratory carcinogenesis.

Proposed Course: Measurement of tracheal cell kinetics (length of S-phase using double labeling with ^{3}H- and ^{14}C-thymidine and labeling index using colchicine and continuous labeling with ^{3}H-thymidine) will be continued on hamsters maintained at different vitamin A levels following benzo(a)pyrene administration. Studies of the effect of benzo(a)pyrene on that subpopulation of cells which labels very lightly with ^{3}H-thymidine will be extended. The variables affecting intracellular benzo(a)pyrene binding will be examined in collaborative studies. DNA repair synthesis in the respiratory epithelium following exposure to benzo(a)pyrene and its metabolites will be studied.

Date Contract Initiated: June 2, 1972

Current Annual Level: $110,000 (Estimated)

V. PUBLICATIONS OF THE CARCINOGENESIS PROGRAM

A. COLLABORATIVE PROGRAM

1. Alarif, A. and Epstein, S.S.: The uptake and metabolism of N(^{14}C) - methyl-N-nitrosourethane and methyl nitrosourea in guinea pigs, and their in vitro metabolism in the guinea pig and human pancreas. In: An IARC Monograph (In Press).

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